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Glial Changes in the Facial Motor Nucleus of the Hamster During Development and After Axotomy

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GLIAL CHANGES IN THE FACIAL MOTOR NUCLEUS OF THE HAMSTER DURING DEVELOPMENT AND AFTER AXOTOMY

by

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VITA

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INTRODUCTION

Nerve cells are known to undergo specific morphological responses to injury and stress. Sometimes these are followed by recovery to normal appearance; sometimes they result in cell death (Lieberman, 1971). These changes vary with the age of the animal and the stage of maturation of the neuron (LaVelle and LaVelle, 1958a, 1958b, 1959, 1975).

The accompanying changes in the surrounding glial cells are less well documented. Long recognized as providing a supportive network for neurons, the glia are now known to be associated with myelination, nutrient transport, and phagocytosis within the central nervous system, but their subtler relations to neuronal development, metabolism and injury are open to considerable question. There is no agreement in the literature on any existing description of a developing glial population, nor is there any clear-cut picture of the glial response to neuronal trauma, especially during development.

The recent interest in the possible interdependence of neuronal and glial metabolism underscores the importance of defining the changes that occur in the glial population during normal neuronal development and after neuronal injury or stress at different developmental stages and in the adult. The purpose of the present work has been to follow the cytological and population changes exhibited by the glial cells of the facial motor nucleus (1) during normal development of the hamster brain and (2) as related to neuronal reactions to severance of the

facial nerve at different ages. An integral part of this project has been the actual identification of each glial cell type as seen in the hamster.

For a study of developing brain, the hamster is particularly useful because its short gestation period results in relatively greater immaturity at birth, with the consequent opportunity provided to affect neurons experimentally over a more extended postnatal range of reactivity. Since the stages of neuronal development, metabolic maturation, and response to injury in the hamster facial nucleus have already been defined (LaVelle and LaVelle, 1958a, 1975), they could, therefore, serve as reference points for the changes that occurred in the accompanying glial development and reactivity. Furthermore, since there was known to be a marked difference between adult and young hamsters in their neuronal response to injury, a different glial response at different ages could be reasonably anticipated.

Using the criteria for glial identification established by others in the field, it has been possible to demonstrate (1) shifts in glial populations during early postnatal development, (2) a stable glial relationship with older neurons, which undergo recovery after a transient chromatolysis following axotomy, and (3) an increased density of the glial population, along with evidence of their phagocytic activity, in response to neuronal death after axotomy at the early developmental ages. The implications of these glial changes, as they relate to neuronal competence for self-maintenance, are considered.

LITERATURE REVIEW

IDENTIFICATION OF THE NORMAL CELLULAR ELEMENTS

Light microscopy of neurons

Many features distinguish nerve cells from other elements in the nervous system. Although neurons vary tremendously in shape and diameter (2-100 microns), they have four main components that are morphologically distinct: the cell body or soma, the dendrites, the axon, and the axon terminals or boutons. For this study, descriptions will be limited to the 'typical,' large, multipolar neurons, such as are found in the facial motor nucleus (FMN).

The most conspicuous histological elements within a motor nuclear area are the neuronal somas. When stained with a basic dye, the cytoplasm is found to contain distinct clumps of basophilic material, the Nissl substance. The appearance and amount of Nissl substance vary with the metabolic state of the cell (LaVelle, 1970; Carpenter, 1976). The soma and dendrites have the highest concentrations of Nissl substance, and the axon hillock area and axon the least. Usually found in the center of the soma is the nucleus, a large, ovoid, vesicular structure containing a single round nucleolus.

Broad-based processes, the dendrites, can be seen extending from the soma in any given histological section, providing the characteristic multipolar configuration. It has been estimated that for multipolar cells of the spinal cord, dendrites represent approximately 80% of the total neuronal surface (Minkler, 1972).

The single axon of a multipolar neuron is thinner than the dendrites and more difficult to identify in light microscopy. The axons of motor neurons, of course, exit to the peripheral nervous system, but axons from many other sources enter the nuclear area to make synaptic contact with the motor cells. Such axons typically have branches (collaterals) along their length and at their termination. Metallic impregnation of nervous tissue with silver reveals the terminals to be cup-shaped or cone-shaped bits of cytoplasm adherent to other neurons. The structure of terminal boutons and of synaptic contacts has been understood only after extensive ultrastructural study.

Light microscopy of neuroglial cells

Virchow (1860) first designated the non-neuronal elements of the central nervous system as 'nerve glue' (neuroglia). Early investigators (see Carpenter, 1976) soon learned that a connective tissue framework, such as is common to other organs, is lacking in the central nervous system. Instead they saw small cells of irregular shape which were regarded as interstitial material. In preparations stained with basic dyes, only the nuclei of these glial cells could be seen, so complex selective staining methods were devised to characterize them further. Impregnation with heavy metals, in particular, showed differences in cell types and their processes.

Based upon their comparative sizes, the neuroglia have been divided into two main groups: the macroglia and microglia. Macroglia can be further subdivided into two classes of cells based on their morphology: the astrocytes and the oligodendrocytes (oligodendroglia). Macroglia are known to arise from the neuroectoderm of the

neural tube, while microglia are classically believed to be derived from mesodermal elements (Arey, 1974; Carpenter, 1976).

The astrocyte is recognized as a stellate cell with many processes extending into the surrounding tissue. With the use of a Cajal gold preparation, some of these processes have been traced to the surfaces of blood vessels, where they are seen to form 'endfeet.' With a Golgi heavy metal preparation, these cells can be found to form a network of processes, in which other neural elements are entwined (Carpenter, 1976). So-called 'fibrous' astrocytes appear to have thinner, less branched processes than 'protoplasmic' astrocytes, and are more readily found in white matter, while the protoplasmic astrocytes are more numerous in the gray matter. The nuclei of both kinds of astrocyte are recognized in basic dyes by their relatively pale nucleoplasm and single, central clumps of chromatin (Ling, et al., 1973).

The term 'oligodendroglia' was introduced by del Rio Hortega (see Penfield's translations, 1932) to describe cells with few processes, which protruded only a short distance from their spherical bodies. In general, the cell body is smaller than either of the types of astrocytes. Seen with basic dyes, the nucleus is usually smaller and rounder, and characteristically denser, than that of the astrocyte, and contains several coarse clumps of chromatin which are often adherent to the nuclear membrane (Ling, et al., 1973).

Based on its location, the oligodendrocyte can be classified as one of three principal types. The first is the perineuronal satellite cell, which is closely apposed to the neuronal soma, most typically found in adult material (Torvik, 1975; Peters, Palay, and Webster,

1976). Secondly, interfascicular oligodendroglial cells are commonly found in the white matter, lying between myelinated fibers. A third type, as demonstrated by a few authors (Cammermeyer, 1966; Luse, 1968), includes the perivascular cells, with processes terminating on blood vessels.

In 1919 del Rio Hortega introduced his silver carbonate staining methods, which disclosed the presence of microglia in both white and gray matter. Seen with this stain, they are small cells which possess abundant short processes with many spine-like projections. With basic dyes, the microglial nulceus is considered to be elongate, narrow, and relatively densely packed with chromatin (Ling, et al., 1973).

Although metallic impregnation techniques have been used to provide the classical descriptions of glial cells, the very selectivity of the stain is such that all cell types cannot be simultaneously viewed in the same histological preparation. This has given rise over the years to problems of correct identification and interpretation. In recent years, with the advent of electron microscopy, identification of neuroglia has been based upon both nuclear and cytoplasmic characteristics of the cells.

Light microscopy of the neuropil

The term 'neuropil' has been assigned to the complex interdigitating 'background' of neuronal tissue, composed primarily of vasculature and the processes of neurons and glia. It encompasses all but the cell bodies of neurons and glia. At the light microscopic level it is almost impossible to identify any of the components except

the blood vessels, primarily their endothelial cells. Yet, it has been estimated that the neuropil is actually 94% of the tissue volume of nuclear groups (Jaeger, Crace, Minkler, 1968).

Electron microscopy of neurons

In large motor neurons Nissl substance remains a distinctive characteristic even at the electron microscopic level and can be seen as stacks of cisternae of rough endoplasmic reticulum (RER). Actually these cisternae anastomose frequently, and this component can be thought of as pervading the entire cytoplasm (Peters, Palay, and Webster, 1976). Ribosomes cover the external surfaces of the cisternae and, in addition, can be found lying freely in the cytoplasm as aggregates or polyribosomes. Their presence primarily as polyribosomes in certain types of nerve cells is translated at the light microscopic level as a diffuse cytoplasmic basophilia.

The agranular reticulum is a prominent component of many neurons. Many workers (see Peters, Palay, and Webster, 1976) have catagorized the various configurations of this organelle. One type of interest here is the subsurface cisternae. These are large flattened vesicles, seemingly attached to the innermost aspects of neuronal plasma cell membranes. An exact function has not been agreed upon for these organelles. Postulations include sites where ions and material could be taken up from the surrounding extracellular area and channelled directly into the reticular system of the neuron (Rosenbluth, 1962).

Also present within the neuron are the common organelles found in other cells, such as mitochondria, Golgi apparatus, and lysosomes.

There are many specific types of vesicles, multi-vesicular bodies and various inclusion bodies common to neurons.

Two elements specific to nervous tissue are microtubules and neurofilaments. These are found in the neuronal perikaryon as well as in the axon and dendrites (Peters and Vaughn, 1967; Torvik and Skjørten, 1971b). It would seem that these organelles run randomly throughout the cytoplasm, yet most investigators (see review by Gray and Guillery, 1966) agree that they provide an orderly structural orientation for the neuron. Neurofilaments are smaller in diameter (100 Å) than microtubules (200-260 Å).

Each large motoneuron has a spherical to ovoid nucleus with a very regular contour to the nuclear envelope. However, smaller neurons and developing neurons have an irregularly creased nuclear envelope. Neuronal nuclei are characterized by a large spherical nucleolus. In mature motor neurons one nucleolus is commonly found (LaVelle and LaVelle, 1958a).

Electron microscopy of neuroglial cells

As a result of ultrastructural studies, the characterization and classification of neuroglial cells have become increasingly complex. Sections particularly of cerebral cortex of adult rodents and primates have been studied by many investigators in an attempt to arrive at consistent nuclear and cytoplasmic criteria for identification of each of the glial elements (Cammermeyer, 1966; King, 1968; Griffin, Illis and Mitchell, 1972; Ling and Ahmed, 1974).

In identifying astrocytes, electron microscopists first used two criteria known from light microscopy, i.e., the processes or endfeet resting upon capillaries and the presence of cytoplasmic fibrils. Both 'protoplasmic' and 'fibrous' astrocytes are now recongized as pale cells with a large nucleus. The karyoplasm possesses low and even density, with little clumping of chromatin material. Fibrils or filaments are the most common cytoplasmic constituent. These filaments occur in tightly packed bundles, unlike the looser distribution of neurofilaments, which they resemble ultrastructurally (Peters, Palay and Webster, 1976).

There are fewer fibrils in the so-called protoplasmic astrocyte than in the fibrous type (Peters, Palay, and Webster, 1976). However, the number of fibrils differs in various regions of the brain, even in the same animal. Among cerebellar astrocytes, designated as Golgi epithelial cells, Palay and Chan-Palay (1974) found cells in which fibrils were absent. No designation was made as to whether these were fibrous or protoplasmic. On the other hand, Scheibel and Scheibel (1955) examined the inferior olivary nucleus and found cells laden with so many fibrils that they chose to call them fibrous astrocytes even though they were found within a nuclear group.

Another feature, found only in the protoplasmic astrocyte, is a highly irregular cytoplasmic contour. Stensaas and Stensaas (1968) reconstructed astrocytes from serial thin sections in order to examine the profiles of their processes. The processes were highly complex extensions, irregular in outline, sometimes seen as sheets of cytoplasm, and rarely seen in section as round in shape. Palay and

Chan-Palay (1974) have shown that the processes of the astrocytes of the cerebellum exhibit extremely slender, veil-like extensions and they therefore called these cells 'velate astrocytes.'

In contrast to other neural cells, astrocytes have a sparse number of typical organelles. The rough endoplasmic reticulum, ribosomes, mitochondria, and Golgi complex are all present, but in small quantities. Overall, the astrocytic cytoplasm has a low electron density.

In an attempt to determine whether fixation alone causes the variability in amount of glycogen seen in nervous tissue, Phelps (1972) studied the use of different types of anesthesia prior to fixation for electron microscopy. He showed large changes in the amount of glycogen seen in astrocytes. It seems that this difference in amount may be a consequence of metabolism of the cell, caused by anesthesia or ischaemic trauma to the cell.

One feature of the astrocyte which seems distinctive to primates is its intimate anatomical relationships with myelinated axons (Ling and Ahmed, 1974). Another feature these workers have shown in the primate is a membrane thickening between adjacent astrocytes, different from that ordinarily identified as a gap junction (Brightman and Reese, 1969). The function of these features has not been firmly established.

The glial cells that offer the most variety of morphological structure are the oligodendrocytes. With the electron microscope these are seen as moderately dense cells that readily contrast with surrounding cells. Their nuclei have been variously shown to be round,

oval, or irregular, but in all cases the nuclear chromatin is seen in coarse clumps (Kruger and Maxwell, 1966; Wendell-Smith, Blunt, Baldwin, 1966).

Most sections of oligodendrocytes show a large mass of cytoplasm at one pole of the cell, indicating that the nucleus is in an eccentric position. Prominently seen in this cytoplasm is RER arranged in small stacks of cisternae. Rosettes of ribosomes, as well as free ribosomes, are commonly found, and the Golgi complex is easily identified throughout the perikaryal cytoplasm (Peters, Palay, and Webster, 1976).

Unlike astrocytes, oligodendrocytes do not have any cytoplasmic fibrils or glycogen granules. They do, however, possess microtubules. These microtubules have a diameter of 250 Å and are similar to those seen in neurons. They are arranged parallel to each other, though they otherwise show no particular arrangement within the perikaryal cytoplasm and can also be found extending into the cell processes (Carpenter, 1976).

Variation in oligodendrocytic morphology was investigated by Mori and Leblond (1970), who reported three classes: light, medium, and dark, based on staining characteristics. The dark type was the smallest and had the most dense chromatin pattern, as well as the most electron dense cytoplasm. A developmental gradient was suggested such that the light oligodendrocytes were the most mitotically active of the cells and became darker with maturation.

One area of concentrated research has been concerned with the identification of microglia. During the 1960's, electron microscopists were involved in a controversy concerning the identification of a glial

cell type in normal central nervous system which could be related to the 'resting' microglia of classical neurohistology. Some authors doubted the existence of such cells in normal nervous tissue at all (Maxwell and Kruger, 1965a; King, 1968). Others (Vaughn, 1967; Vaughn and Peters, 1968) found, in normal adult rat optic nerves, a glial cell type morphologically distinct from astrocytes or oligodendrocytes but designated as a 'third type' of neuroglia, rather than as microglia. Described as smaller than either of the macroglial cell types, this possessed short, stout protrusions from the perikaryal cytoplasm. Neither the fibrils and glycogen common to the astrocyte, nor the microtubules common to the oligodendrocytes, were seen within the cytoplasm of this cell. Also, in normal tissue such cells were found to contain certain dense laminar bodies and lipid droplets in their cytoplasm.

This 'third type' of neuroglia was still not directly comparable to the classical microglia of del Rio Hortega (1932), nor was its ultrastructural description in agreement with the early electron microscopic observations which described a small, very dark cell with an equally dense cytoplasm with irregular contours (Farquhar and Hartmann, 1957; Mugnaini and Walberg, 1964). Most authors now agree (Vaughn and Peters, 1968; Peters, Palay, and Webster, 1976) that these dark cells of early electron microscopy represented profiles of poorly fixed cells, or possibly small neurons of tissue that was improperly handled during preparation. Another factor lending credence to the presence of an early fixation problem is the fact that, in time, as improved methods of fixation were more commonly used, reports of these

cells diminished in frequency.

Mori and Leblond (1969) studied the cerebral cortex of the rat with a view to determining what the silvered cells of del Rio Hortega were like ultrastructurally. Their protocol involved staining the cortical sections with silver, with the expectation that since the silver was electron dense, it could provide contrast for ultrastructural analysis. By combining serial thick and thin sections, they observed the classical microglial cells ultrastructurally with clarity, and chose to call them 'microglia' even though the description was similar to that for the 'third type' of neuroglia described by Vaughn and Peters (1968). Furthermore, Mori and Leblond (1969) classified microglia into two types based on location within the tissue: the interstitial microglia lying randomly throughout the neuropil, and a pericytal type, which lay near the blood vessels. Other authors have described as 'microglia' certain cells similar in description to the 'third type' of neuroglia of Vaughn and Peters (1968), in normal as well as pathological tissues (Blinzinger and Kreutzberg, 1968; Barón and Gallego, 1972; Torvik, 1972).

A somewhat limited agreement concerning the morphology of normal microglia has now evolved. The microglial cell is usually smaller than either the astrocyte or oligodendrocyte and possesses none of the specific cytoplasmic components of the astrocyte. Its nucleus most closely resembles that of an oligodendrocyte, though its chromatin is more prominent and coarsely clumped. The electron density of the microglial cell is similar to that of the dark oligodendrocyte and it possesses a few microtubules in its cytoplasm. A reported difference

in the microglial cytoplasm is the narrow or stringy configuration of the cisternae of the RER (Peters, Palay, and Webster, 1976). Still another feature of the microglial cytoplasm is the presence of dense inclusion bodies or lipid laden bodies (Cammermeyer, 1970; Baron and Gallego, 1972). It should be noted that the above description also fits the 'third type' of glial cell.

The ultrastructural similarity between oligodendrocytes and microglia is not surprising in view of the fact that, with some modification of del Rio Hortega's classical techniques of silver staining, both oligodendrocytes and microglia cell types can be impregnated in the same section and viewed simultaneously (Cammermeyer, 1966, 1970). Usually similarities in staining abilities suggest a similar chemical property. It is plausible to suspect that these cells may be developmentally related (Carpenter, 1976).

NORMAL DEVELOPMENT OF THE CELLULAR ELEMENTS

Neuronal development

The neuroblast undergoes differentiation both during and after its migration to its final destination (Caley, 1971). When first recognized, it has an elongate nucleus, with multiple nucleoli and a scant amount of cytoplasm surrounding the nucleus. Many studies have examined the morphology of the neuroblast as it differentiates (Shimada and Langman, 1970; Caley, 1971; Rakic, 1972), and they have revealed a composite of events that occur in all parts of the cell. The cytoplasmic organelles increase in number and complexity. Most noticeably, there is a shift from scattered polyribosomes to a

prominent stacking of RER (Caley, 1971). With light microscopy, this is seen as a change from a dispersed basophilia to clumps of basophilic material called Nissl substance (LaVelle, 1951, 1956).

In detailed sequential studies of developing facial motor neurons of the hamster, LaVelle and LaVelle (1958a, 1958b, 1959) showed a close time correlation between these cytoplasmic changes and developmental changes in the nucleus. Major developmental changes took place at quite specific ages. By three days before birth in the hamster the cells of the facial motor nucleus (FMN) have already migrated to their ultimate position and form a distinct group of cells. They are morphologically immature, with multiple nucleoli and scanty cytoplasm. Between birth and 7 days postnatal age many changes occur, including development of the Nissl pattern to its adult configuration. At this same time, the cell attains a single, large nucleolus with a definitive distribution of RNA and DNA moieties.

Most changes seen at the light microscopic level after this time period involve increases in size of the cell, particularly in the cytoplasm, with a more moderate increase in volume of the nucleus. More recently, in a study of the nucleoli of injured facial motor neurons of hamsters, LaVelle and LaVelle (1975) have described an intranucleolar body (INB) which first develops between 20 and 25 days postnatal age. It is viewed as morphological evidence of the maturation of the cell's ability to produce proteins.

Accompanying these morphological events are many biochemical changes, including enzymatic ones, which cause and govern the morphological changes. Many problems arise in an attempt to correlate precise

morphological differences with the biochemical ones. Differentiating systems, however, are difficult to examine biochemically because of the fragility of the cells. (See section on functional relationships of neurons and glia.)

Glial development

Most textbooks (Langman, 1969; Arey, 1974) state that, after neuronal migration has begun, the macroglia begin to proliferate from undifferentiated cells of the neuroepithelium. These undifferentiated cells give rise to spongioblasts, which then become either astrocytes or oligodendrocytes. Many intermediates in this differentiation process have been identified with the electron microscope. Immature forms of astrocytes and oligodendrocytes are distinguished by their resemblance to their mature forms (Caley and Maxwell, 1968b; Vaughn and Peters, 1971). The cells of the oligodendrocytic line have an oval nucleus with clumps of chromatin located throughout the nucleus, whereas the nucleus of developing astrocytes is paler, with a single prominent chromatin clump. Throughout maturation there is an increase in cytoplasmic mitochondria, ribosomes and microtubules (Caley and Maxwell, 1968b; Vaughn and Peters, 1971; Ling and Leblond, 1973; Ling, et al., 1973).

It is well accepted that astrocytes and oligodendrocytes are derived from a common stem cell within the neuroepithelium and are, therefore, ectodermal in origin (Caley and Maxwell, 1968b; Vaughn and Peters, 1971). Until recently microglia have always been considered to be mesodermal in origin, due to the classical work of del Rio Hortega

(see Penfield's translation, 1932). It was held that these cells arose from undifferentiated cells in the connective tissue surrounding the invading blood vessels that supply the brain. Recent investigators, however, have proposed that the microglia are derived from the neuroepithelium (Lewis, 1968; Blakemore, 1969; Ling and Ahmed, 1974; Stensaas, 1975). Blakemore (1969) has presented the theory that an undifferentiated stem cell can give rise to either macroglia or microglia. As mentioned earlier, some investigators today deny the existence of microglia as a distinct glial entity altogether (Kruger and Maxwell, 1966; Caley and Maxwell, 1968b; Vaughn and Peters, 1968). Vaughn and Peters (1971) have suggested that the 'third type' of glial cell is a further differentiated oligodendrocyte.

NORMAL FUNCTIONS OF GLIAL ELEMENTS

Despite much recent work, there is still a great deal of uncertainty concerning the functions of the various glial cells. It is striking that many of the recent hypotheses are simply modifications of the older theories. One generalization can be made: these cells are not static in normal tissue; they perform many known functions and, as a better understanding of the limitations of the neurons themselves is gained, perhaps even more can be attributed functionally to the glial cells.

In 1865 the concept that <u>astrocytes</u> are the structural supporting elements within the central nervous system was emphasized by Weigert (see Peters, Palay, and Webster, 1976). Although no direct evidence for this theory has been accumulated, most textbooks still list support

as an astrocytic function. Certain features or specializations of astrocytes suggest a supportive function. Through their extensive attachments to blood vessels and their contacts at the glial limitans, astrocytes can be said to cover all the exposed surfaces of the central nervous system. Also, the astrocytes form membrane thickenings at their junctions; in invertebrates and amphibia actual desmosomes can be found between astrocytes (Kuffler and Potter, 1964; Kuffler, et al., 1966). These neuroglia from lower animals are said to participate in a functional syncytium, so the analogy can perhaps be drawn to the mammalian system.

One early hypothesis that has remained for decades regards the participation of astrocytes in the blood-brain-barrier. For many years workers have searched for an anatomical barrier. Again, the presence of astrocytic endfeet adjacent to capillaries seemed to indicate the role of a barrier. The most recent evidence, however, fixes the site of the barrier at the tight junctions between the vascular endothelial cells (Brightman and Reese, 1969).

Related to the barrier hypothesis, astrocytes have been implicated in a transfer or transport function. The idea was first suggested as a result of electron microscopic observations (deRobertis and Gerschenfeld, 1961). Later, enzyme studies (Adams, 1965) suggested that astrocytes may be involved in certain mechanisms of transport between the blood vessels and the neurons. Because of the large surface area of the neurons that is covered by astrocytic processes, Hydén (1967) stated that such contacts may provide sites of metabolic exchange between neurons and astrocytes. Considering all the proposed theories, most

textbooks suggest that the astrocytes play a major role in the regulation of flow of metabolites in the CNS.

Almost a century ago, Cajal put forth the concept that glial cells participate in isolating neurons or groups of neurons within the CNS (see Peters, Palay, and Webster, 1976). It has only been with recent electron microscopic studies that this theory could be verified. The patterning of astrocytic processes has been found to vary in different parts of the central nervous system, but the net result in each instance is usually the segration of, or isolation of, the receptive surfaces of neurons (Peters and Palay, 1965; Palay, 1966). For example, synaptic glomeruli in the thalamus are surrounded by astrocytic sheets. Axon terminals synapsing on the anterior horn cells of the spinal cord are isolated into groups by astrocytic processes. Peters and Palay (1965) indicated that the astrocytes thus promote localized performance of the terminals by preventing the spread of their influence to other, non-related neurons. The recent extensive electron microscopic observations of Chan-Palay (1972) on astrocytes indicate that the processes of velate astrocytes segregate or isolate the other components of the cerebellum.

The primary function of the oligodendrocyte is formation and maintenance of the myelin sheath of neuronal processes. Evidence for this role is based upon several observations. Oligodendrocytes first appear in tracts of the brain close to the time of myelination (Vaughn, 1969) and studies have shown an increased mitotic division of these cells immediately preceeding myelin formation (Hirose and Bass, 1973). Also, an increase in the rate of glial enzyme activity has been found

just prior to myelination (Friede, 1961). Direct connections have been seen in developing tissue between oligodendrocytes and newly forming myelin sheaths (Bunge, Bunge and Pappas, 1962; Bunge and Glass, 1965).

Curiously, there has been no demonstration of continuity between the oligodendroglial cell and myelin sheaths in normal mature tissue. Attempts to demonstrate this with electron microscopy have been made in several laboratories. The lack of anatomical evidence has been attributed to the thinness of the processes of oligodendrocytes and to the need to trace these processes with serial sectioning techniques (Peters, Palay, and Webster, 1976). Actually, because of this lack of evidence the role of oligodendrocytes in the maintenance of the mature myelin sheath may be in question.

The observation that oligodendrocytes often are seen in a perineuronal position has lead investigators to suggest that these cells play a role in the nutrition or maintenance of neurons (Peters, Palay and Webster, 1976). Many studies have utilized biochemical methods of analyzing the content of oligodendrocytes and neurons in order to assess the existence of intricate interrelationships. Hydén (1976) and his collaborators dissected the neurons of Deiter's nucleus and their surrounding glial ensheathments from the rest of the CNS. They found a difference in oxidative enzyme levels and composition of nucleic acids in these neurons and their surrounding glia. Hydén feels that this imbalance in metabolism indicates a symbiosis between the two cell types. One criticism of his conclusions of importance here involves an electron microscopic study of Deiter's nucleus (Sotelo and Palay, 1968), in which oligodendrocytes were found only rarely to contact the

neuronal surface.

A function commonly attributed to the microglial cells is phagocytosis of debris in the CNS. Much of the literature on the functions of microglia is confusing, and it remains difficult to interpret since it is concerned not only with microglia in normal tissue but with the response of these cells to various experimental and pathological states.

Some investigators have considered that in the normal adult the so-called microglial cells may actually represent a multipotential cell which, like the multipotential cells of other tissues, remains inactive until stimulated to form new cellular elements as the need arises (Vaughn and Peters, 1968, 1971; Vaughn, 1969). These new elements may be astrocytes, oligodendrocytes or additional multipotential cells.

While it might seem a step in the wrong direction to examine thinner sections and smaller samples of tissue in order to assign functions to neurons and glia, actually it is crucial to review the ultrastructure. Many authors agree that for an understanding of neuronal-glial relationships, electron microscopy offers advantages not found in the traditional techniques using basic dyes or silver impregnation (Palay, McGee-Russell, Gordon and Grillo, 1962). One advantage is that the nuclei, somas, and processes of all the different cells can be viewed within a single preparation, so they can be readily compared. Also, the membranes of each cell type are visible, and can be seen in apposition to other structures.

The development of cell separation techniques has enabled investigators to study further the possible functional interrelationships of neurons and glial cells. By isolating the neuronal and glial

populations of the brain, investigators can determine what is peculiar to each cell type (Rose, 1965; Blomstrand and Hamberger, 1969; Norton and Poduslo, 1970; Johnson and Sellinger, 1971).

These separation procedures, coupled with the use of radioactively labelled amino acids, have provided a profile of protein synthesis in the normal neuronal and glial populations of adult animals. Many reports have stated that glial cells take up amino acids at a greater rate than neurons, but that the actual incorporation of these amino acids into proteins is greater in neurons than in glia. It has been suggested from this information that glia may play a role in the regulation of neuronal protein synthesis by controlling the level of extraneuronal amino acids (Hamberger, 1971; Lisý and Lodin, 1973; Blomstrand, et al., 1975).

These techniques can also provide information on RNA synthesis in both cell populations. Jarlstedt and Hamberger (1971) incubated uridine with cortical slices from adult rabbit brain and then separated the neurons and glia and measured total RNA synthesis. They showed a nearly equal rate of total RNA synthesis for the first hour of incubation with the isotope, followed by an increased neuronal synthesis after 3 hours of incubation. This suggests a possible transfer of some RNA from the glia to the neurons. In a recent piece of work on RNA synthesis, involving a specialized separation procedure, Løvtrup-Rein (1970) and Løvtrup-Rein and Grahn (1970) have claimed to be able to isolate the nuclei of neurons, astrocytes, oligodendrocytes and microglia from the whole cerebral cortex of the adult rat. They reported specific RNA's in each of the different populations and suggested that this may reflect a functional specialization of each cell type.

Methods of isolation of cell types from brain have come under great criticism (Watson, 1974). Good methods for obtaining a high yield of glial cells of a single type have not proven satisfactory. Even when a certain type of glial cell is thought to predominate in a given area, it is difficult to obtain a 'pure' fraction. A common error made in this field is to presume that the glia of white matter are almost entirely oligodendrocytes, yet morphologically (depending on the area of the brain and species examined) astrocytes are usually described as comprising 25% of white matter (Watson, 1974), Nevertheless, biochemical observations of fractionated cells continue, as techniques for better separation are worked out in many laboratories.

In a recent summary of his work in progress, Rose (1976) described his improved methodology for bulk separation of fractions of cerebral and cerebellar cortex. With electron microscopic observations of these fractions, he has shown purified populations of specific cellular types. He examined many biochemical criteria and concluded that neuronal and glial metabolism is "state-dependent." By this he meant that the metabolism fluctuates with the changing environmental circumstances of the organism. He further explained that the dynamic biochemical interrelationships between neurons and glia are, at the cellular level, correlates of the plasticity of neuronal function at the physiological level.

Electrophysiologists have long recorded the membrane potentials of neurons in an effort to determine a basis for neuronal activity (Minkler, 1972). More recently the glial cells have been included in a study of membrane potentials. After examining non-neuronal cells identified as Mullers fibers in the fish retina, Svaetichin, et al. (1965) proposed

calling them 'controller cells' since they interacted with receptors and neurons in a specific manner. Because these non-neuronal cells possessed electrical characteristics different from neurons, the investigators proposed that nervous function is based upon an interaction between the neurons themselves and the non-nervous elements. The neuronal elements function according to classical concepts, while the non-neuronal exert both excitatory and inhibitory control of the neurons. The reciprocal inhibition-excitation system of the fish retina results in wavelength-dependent firing patterns for the neuron; this system, however, is dependent upon the non-neuronal controller system.

A recent study of membrane potentials in glial cells of the cat cortex has provided information on the sensitivity of neuroglial cells to the ionic flow of neural tissue (Futamachi and Pedley, 1976). Previous experiments on amphibia (Kuffler and Nicholls, 1966) showed that the glial cells had high resting membrane potentials with sensitivity to potassium concentration gradients, absence of spike generations, and low resistence interconnections. All of these properties have led to the view that in amphibia the glia function in a syncytium. The investigations of the cat cortex showed that mammalian glia are similar to those in lower animals, with the exception that their membranes are sensitive to ions in addition to potassium. Although direct physiological evidence is lacking for a glial syncytium in mammalian cortex, recordings made on glial cell bodies have reflected potassium changes occurring at sites remote from the cell bodies in question, thereby suggesting the possible existence of a syncytial arrangement. Other electrophysiological studies (Ransom, 1974) have shown that in recordings at the cortical surface the depolarizing

response is due to electrical shifts in the glial cells rather than in the neurons. Much current work on this electrical activity is centering around epileptic and epileptogenic sites in the cortex as related to concurrent glial ionic sensitivities (Ransom, 1974). As more electrophysiological data are presented, more speculations can be made concerning a possible integrative function of glia in neural conduction.

Galambos (1965) has suggested that the glia may play a role in synaptic transmission. He based his view largely on the appearance of numerous close contacts between glia and neurons at synapses and the fact (Kolle, 1962) that glia contain large quantities of non-specific cholinesterase.

Considering the probable functional interrelationships of neurons and glia, an interesting point of view that has been emphasized by Watson (1974) is the comparison between the nervous system and epithelium. Ependymal cells and astrocytes exhibit some typical epithelial features, such as the formation of a basal lamina and intercellular contacts by gap junctions. Some epithelial functions performed by these cell types include regulation of the size and shape of the cellular compartment, as well as regulation of migration and differentiation of neurons and other glial elements. Watson (1974) states that oligodendrocytes are not characteristically epithelial in either appearance or function. He summarizes that neurons themselves may resemble epithelial cells only in that they are specialized and highly differentiated, while the glia more readily function as epithelia.

REACTIONS OF CELLS TO EXPERIMENTAL ALTERATIONS OF THE CENTRAL NERVOUS SYSTEM

Following the work of del Rio Hortega (1932) and his identification of cells, it was generally accepted that most of the phagocytes found within pathological brain or cord tissue were glial cells, specifically the microglia. It has become evident, however, in the past few decades that a variety of cell types, both intrinsic and extrinsic to the central nervous system, may participate in phagocytosis in various pathological states of the brain and cord. Many investigators have experimentally injured the central nervous system in laboratory animals as a means of observing shifts in cell populations, as well as changes in cell morphology, during a pathological state. Experimental injuries have included stab wounds to the cortex, tract lesions, peripheral nerve axotomy, enucleation of the eye, irradiation, and exposure to virus particles. Such techniques provide known insults which can be controlled very carefully.

Many investigators have reported the extrinsic origin of CNS phagocytes. Maxwell and Kruger (1965b) studied rat cerebral cortex with the electron microscope following irradiation with alpha particles and obtained results indicating that the vascular pericytes have phagocytic capabilities. In a study on rat optic nerve during Wallerian degeneration (Vaughn and Skoff, 1972), pericytes as well as advential cells were seen as phagocytes. Konigsmark and Sidman (1963) used a variety of neural lesions in their study of the origin of brain macrophages. Based on autoradiographic findings, they suggested that two-thirds of the brain macrophages were of extrinsic, probably hematogenous, origin.

Autoradiography had led other authors to conclude similarly that circulating blood leucocytes play an important role in this process of phagocytosis (Schultz and Pease, 1959; Bignami and Ralston, 1969; Adrian and Smothermon, 1970; Stenwig, 1972; Young, 1977).

Axonal damage by crush or cut lesions results in proliferation of small cells closely associated with the cell bodies of the injured neurons. This proliferative activity has been observed in cells which were labelled by tritiated thymidine injections shortly before sacrifice. There is no doubt that DNA synthesis and mitotic divisions occur in these cells, but whether this increase in non-neuronal cells is due to proliferation of intrinsic or extrinsic elements is open to question. They have been variously identified as glial satellites, juxtavascular histiocytes, microglia, and mononuclear leucocytes (Cammermeyer, 1963a, 1963b; Sjøstrand, 1965a, 1965b; Konigsmark and Sidman, 1963; Kreutzberg, 1966; Freide and Johnstone, 1967; Adrian and Smothermon, 1970; Watson, 1974).

The nerve cell body is necessary for the maintenance of the axon. When the axon is cut, the nerve cell body usually undergoes changes in morphology, metabolism and activity. The morphological changes in the cell soma were first recognized by Nissl in 1892 and have been the subject of many recent reviews (Cragg, 1970; Lieberman, 1971, 1974; Grafstein, 1975).

These characteristic structural changes include swelling of the cell, migration of the nucleus to an eccentric position and the apparent disappearance of basophilic material (Nissl substance) from the cytoplasm. This lack of Nissl substance in particular has led to

the application of the term 'chromatolysis' for the response to axotomy. Investigations of many different types of neurons led to the discovery that chromatolysis as such (i.e., loss of stainability of Nissl substance) does not always occur during the response to axotomy. Other terms have been employed, such as 'axon reaction,' 'retrograde reaction,' and 'cell body response' (Grafstein, 1975).

Variability in the response to axotomy may be understood if the metabolism of the reactive cell is examined. One cell may show a response associated with catabolic activities, while other cells may be undergoing anabolic processes. How much the cellular machinery responds by breaking down cytoplasmic constituents or by mobilizing to form new cytoplasmic structures may account for the differing appearance of cells undergoing 'chromatolysis' (Grafstein, 1975).

While cellular swelling has been a most frequently reported characteristic, the cause for this change is poorly understood. In the early stages of the response the swelling involves an increase in water volume, resulting in a dilution of the cellular contents (Lieberman, 1971; Grafstein, 1975). Too often care has not been taken to distinguish this kind of swelling from increases in size due to augmented dry mass, which is seen later in the response as new cytoplasmic material is stored. In his review of the axon reaction Lieberman (1971) demonstrates the failure of most early workers to determine accurately the extent of the cellular swelling they observed. The range of values from those who attempted to quantify their data is very wide. Cell swelling reported in terms of the percentage volume increase has ranged from 30% in cat spinal motor neurons (Barr and

Hamilton, 1948) to as high as 160% in rabbit hypoglossal neurons (Brattgard, et al., 1957) to as low as 17% in mouse facial motor neurons (Torvik and Heding, 1967). On the other hand, reports of no significant increase of cell size include studies on the same groups of neurons within the same species: cat spinal motor neurons (Schadé and Van Harreveed, 1961), rabbit hypoglossal neurons (Hudson, et al., 1961) and mouse facial motor neurons (Cammermeyer, 1963a, 1963 b). The wide variety of response is puzzling.

The significant water movement that causes swelling of the soma may be due to a membrane depolarization which occurs at the site of axon severance in response to the mechanical trauma. This depolarization leads to an overall lowering of the membrane potential and, thus, to alterations in ionic flow (Grafstein, 1975).

The changes in neuronal membrane may also contribute to a local change in the appositional patterns of cell somas. Some authors have described the displacement of synaptic boutons from the soma of injured neurons (Blinzinger and Kreutzberg, 1968; Sumner and Sutherland, 1973; Chen, 1978). Furthermore, the perineuronal glial cells have been observed to interpose cytoplasmic flanges between the neuronal soma and the displaced boutons (Blinzinger and Kreutzberg, 1968; Torvik and Söreide, 1975; Chen, 1978).

Morphological changes within a reacting neuron include displacement of the nucleus and changes in nuclear size and shape. A shift in the position of the nucleus, from central to eccentric, has been frequently reported by investigators using many animal models (see Lieberman, 1971). The significance of this shift is still unknown.

Both increases and decreases in nuclear volume have been reported following nerve lesion. Investigators obtaining an increase in nuclear volume attribute it to the anabolic activity within the cell. Increases in the folding of the nuclear envelope have been documented in light and electron microscopic studies (Lieberman, 1971).

To some histologists, the nucleolus is used as an indicator of changes in the functional state of the cell. Changes in size, shape and position of the nucleolus have been reported (see review by Lieberman, 1971) following axotomy. Some of these changes may be artifactual (Cammermeyer, 1963a, 1967), but such shifts as 'paling of nucleoli' or an increase in spaces between the nucleolemma seem well documented (Lieberman, 1971, 1974). Observations at the ultrastructural level have shown eccentricity of the nucleoli of chromatolytic neurons (Watson, 1966; Barron, et al., 1971).

Quantitative data on nucleolar size are scarce, but volume increases of 30-50% and up to several hundred percent its own volume have been found (LaVelle and LaVelle, 1958a, 1958b; Lieberman, 1968; Murray and Grafstein, 1969). Most workers have attributed nucleolar volume increases during axon reaction to increased synthesis of nucleolar RNA and, therefore, to an increased content of nucleolar RNA and protein (LaVelle and LaVelle, 1958b; Murray and Grafstein, 1969).

Such major changes in an organelle which functions to synthesize RNA and protein indicated the relevance of examining the RNA and protein content of chromatolytic neurons. The evidence from these metabolic studies reflects the anabolic activity of these injured cells. Axotomy is followed by an increase in nuclear RNA synthesis, nucleolar RNA

content, and an increased rate of passage of nuclear RNA to the cytoplasm, yielding an increased cytoplasmic RNA content (Lieberman, 1971, 1974; Grafstein, 1975). Eventually an increase in cytoplasmic protein synthesis and content can be detected.

This increase in overall protein synthesis and content has been the stimulus for many biochemical studies. From these a most important fact is made apparent: that while the general level of protein synthesis increases, the content of certain specific proteins decreases. Most of the identified proteins which decrease in content seem to be associated with synaptic transmission (Cheath and Geften, 1973). While many workers explain this disproportion as leakage from the site of injury, it is obvious that the response to axotomy stimulates the synthesis of certain specific materials within the neuron. A shift from the production of transmitter-associated materials to materials associated with structural repair and maintenance might be expected (LaVelle and LaVelle, 1975). In an electrophoretic study of protein profiles in normal and axotomized neuronal groups, Griffith and LaVelle (1971) have shown similarities in the protein profiles of chromatolytic neurons and immature neurons which are still changing structurally.

Actually, there is probably relatively little leakage of cell contents at the site of axotomy. Although it might seem that a fairly large portion of cell membrane is lost following axon section, the defect produced in the cell has been described as relatively small. The hole in the membrane of the neuron is first clogged with exoplasmic contents and eventually the membrane reforms (Lubinska, 1964).

It is of interest that the injury itself evokes a burst of action potentials in axotomized neurons. Although this electrical activity is calculated to be very brief in duration, the regenerating axon may develop a continuous action potential which could be the drive behind the elevated levels of synthesis within the injured cell (Evans and Saunders, 1974).

A consistent change seen ultrastructurally in the cell bodies of chromatolytic neurons is a marked increase in number and size of lysosome-like dense bodies (Matthews and Raisman, 1972). An increase can also be noted in the non-Golgi smooth ER, probably related to the formation of these lysosomes. Chemically an increase in cytoplasmic acid phosphatase is found (Matthews and Raisman, 1972).

As already indicated, axotomy results in a wide variety of responses ranging from a transitory morphological change to total cell disintegration. Experiments have indicated several different factors which can influence the response to axotomy.

One important factor is the site of the severed axon, itself, whether it is contained wholly within the central nervous system or lies peripheral to it. Very few studies have been made involving neurons which lie wholly within the brain or cord, but it is clear that such cells respond differently from the neurons of cranial nerves or spinal ganglia (Barron, et al., 1971; Durica and Jacob, 1978).

There is an extensive literature demonstrating evidence that axon lesions close to the cell body produce a more intense and prolonged chromatolysis than more distal cuts and are more likely to result in cell death (see review by Lieberman, 1971, 1974). It is probable that

the more rapid onset of perikaryal responses in neurons injured close to their cell somas mirrors the shorter distance to be covered by the signal or stimulus for chromatolysis (Cragg, 1970). In a series of experiments spanning several years, Watson (1968, 1969, 1970, 1972) used quantitative microchemical methods to provide information concerning sites of injury. He found that the most rapid onset of the nucleolar response, as well as the most rapid termination, followed axon lesions close to the cell bodies of origin of the hypoglossal nerve. To obtain this comparison, he sectioned some hypoglossal nerves at the base of the skull, and others at their point of emergence into the tongue. Axotomy at this distal site produced the longest latency, yet most prolonged response (Watson, 1974).

The reactions of glia to experimental injuries of the brain are sometimes difficult to equate with the known glial responses described in medical pathology. The pathology of the nervous system is especially complex for many reasons, the most important being the anatomical variability of the cellular structures which reside there. The participation of glia in injury and repair does not exclude the participation of those other elements which operate in injuries to the rest of the body. Polymorphonuclear leucocytes, lymphocytes, plasma cells, macrophages and blood vessels have the same role in disease of the nervous system that they have elsewhere. In known diseases of the central nervous system the glia are specialized elements, each serving a specific function (Foley, 1974).

The reactions of astrocytes to insult are catagorized in pathology as regressive or progressive. The regressive type of reaction continues

until the cells become necrotic and disintegrate, while the progressive response involves a glial scar formation or diffuse fibrous gliosis (Greenfield, 1963).

A less complex response seen in astrocytes is neuroglial hyperplasia, which may be stimulated by many factors. Syphilis and some forms of viral infections are associated with a proliferation of astrocytes (Greenfield, 1963). Edema and minor ischaemic trauma are associated with astroglial swelling. Indeed, regressive changes in astrocytes are seen to occur very rapidly within the first few days of an ischaemic trauma. The cells exhibit an irregular cell body, with broken processes, and the cytoplasm may show lipid granules. These cells were called amoeboid neuroglial cells by Alzheimer (1910, see Greenfield, 1963). However, there is no supporting evidence that these reactive cells possess the capacity to migrate. Regressive changes result from the incapacity of the astrocyte to survive the trauma; eventually the astrocytes disintegrate or 'regress' completely.

Progressive changes (Greenfield, 1963) include an enlargement of the cell body but no loss of processes. The glia are said to be stimulated to divide amitotically, resulting often in large bi-nuclear cells. Such cells are often described as giant astrocytes; some have been reported to be 25 microns in diameter. It is interesting to note that these cells may at this point either die and disintegrate or continue their response by forming new processes. The formation of processes by the astrocytes also results in the formation of a so-called 'scar.'

Much of the literature on the oligodendroglial response to brain pathology focuses on their involvement in the demyelinating diseases. However, some generalizations can be made on their cellular pathology (Greenfield, 1963). Oligodendrocytes swell rapidly in response to almost any kind of toxic or metabolic change. Some regard this as the most sensitive indicator of disease of the CNS. This 'acute swelling' is a degenerative change in the cells; they may recover to their normal size, or they may die. Sometimes they seem to increase in number in their perineuronal position, when the neuron itself is damaged; this is called 'satellitosis.'

Microglia have long been regarded by pathologists as histiocytes and, therefore, part of the reticuloendothelial system. They respond most rapidly to stimuli by undergoing hypertrophy and multiplication (Greenfield, 1963). Their principal function has been described as phagocytosis. They seem to be 'less vulnerable' than other neuroglia because they remain very active in areas of necrosis (Greenfield, 1963). They are said to have several reactions or levels of response to injury, including proliferation, hypertrophy, phagocytosis of lipid, neuronophagy and dendrophagocytosis.

The most generalized response of microglia (Greenfield, 1963) seems to be proliferation. This may be diffuse, yet is sufficient for pathologists to identify it as an increased cellularity. Examples of this response are the so-called 'glial stars': clumps of 10-20 microglial cells around blood vessels. This unusual appearance is seen commonly in viral diseases.

One response which bears some scrutiny is that of hypertrophy. Pathology texts (see Greenfield, 1963; Foley, 1974) describe the cell body as irregular in shape and cell nuclei 'lobulated' or 'horse-shoe shaped.' These descriptions of cellular morphology seem to more closely resemble monocytes and neutrophils, raising doubts that they are truly microglia.

During necrotic trauma, such as Wallerian degeneration, the microglia phagocytize lipid. The cell body enlarges with accumulated lipid so that it is called a lattice cell; other names given to this reactive cell type include 'compound granular corpuscle' (Russell, 1963) and 'gitter cell' (Blinzinger and Kreutzberg, 1968). Texts indicate that these cell types are migratory and able to move through the necrotic areas to the blood vessels.

The most extreme microglial response involves the phagocytizing of parts or all of a degenerating neuron. This is commonly seen in viral diseases as well as in carcinomas. It is also seen in areas of chronic ischaemic trauma resulting in neuronal death. As seen in light microscopy, the microglia surround the dying neuron. Ultrastructurally their processes can be seen to be adjacent to those of the dying neuron (Greenfield, 1963; Blinzinger and Kreutzberg, 1968).

MATERIALS AND METHODS

Care and breeding of hamsters

The golden hamster, *Mesocricetus auratus* (Engle, Farmsburg, IN) is well suited for use in a developmental project because of its prolific breeding capacity and short gestation period (16 days) and the relative immaturity of its postnatal young. In this study adult animals were housed singly in plastic cages. Absorbent excelsior (AMXCO, Lombard, IL) covered the floor of each cage. Purina Rat Chow and water were given to the animals *ad libitum*. In addition, one drop of Poly-Vi-Sol (Mead Johnson, Evansville, IN) was administered to each animal each week. The light and dark regimen in the animal quarters was set for a 12:12 cycle.

The estrous cycle of female hamsters is four days in length and was monitored by examining the vaginal discharge daily. In the morning following estrus a thick, caseous discharge could be extruded from the vagina. Once her cycle was established, a female was placed in the male's cage the evening before estrus. The female was observed for signs of lordosis, and the initial mating was observed. The following morning the animals were separated and the date of mating and expected date of birth were recorded. At least five days prior to the expected parturition, the female was given additional food within the cage and paper strips for nesting purposes. Parturition normally occurred 16 days after the mating, and the young were considered to be 'new born'

during the first 12 hours after birth. By 24 hours after birth they were considered to be 1 day old.

Anesthesia

Prior to either axotomy or perfusion, hamsters were anesthetized with an intraperitoneal injection of Nembutal (sodium pentobarbital 50 gr/ 1cc). The dosage administered was dependent on the age and weight of the animals and was as follows: adults (.16 ml/ 100 gr), 20-day (.033 ml/ 25 gr), 15-day (.021 ml/ 15 gr) and 10-day animals (.016 ml/ 10 gr). Newborn and up to 5 day-old animals were anesthetized by chilling on ice.

Axotomy

Axotomy of the right facial nerve was performed on hamsters of both sexes at the following postnatal ages: 1, 5, 10, 15, and 20 days old and adult. The postnatal ages were chosen to correlate with major developmental states of maturation of the brain of these animals. Adult animals were at least 90 days old and weighed between 100 and 180 grams. The left facial nerve in each animal remained intact and served as a control.

The anesthetized animal was placed on a small, operating platform, and the area immediately anterior-inferior to the external ear was shaved and cleansed. The skin in this area was then reflected, and an A-O dissecting microscope was used to facilitate location of the facial nerve at its exit from the stylomastoid foramen. Upon being located, the nerve was dissected distally until it branched into its four main branches. The nerve was cut with irridectomy scissors

at its point of emergence from the skull, as well as at points distal to the branching (each branch was cut). The intervening segment was then removed, thus preventing the facial nerve from reestablishing its peripheral connections. The wound was closed with stitches (cotton thread) in the four youngest age groups, and with metal wound clips in the 15 day and older animals. The animals were returned to their cages for postoperative periods of time as follows: 4 days, 10 days, 20 days, 50 days and 100 days for each age group. At least 3 animals were used for each survival time in each age group. A total of at least 150 animals were operated upon. Approximately 110 animals were used for the light microscopic investigation, while the remainder were used in the ultrastructural analysis.

Postoperative care and observations

One problematical aspect of this project involved returning the operated pups to the mother's nest. Because of a high incidence of cannibalism in hamsters, extra care was warranted. All pups were allowed to recover fully from anesthesia before being returned to the nest, and wounds were cleansed with alcohol (50%) for removal of any blood. Extra strips of paper with some alcohol on them were introduced into the nest prior to the return of the pups. The youngest animals were warmed in the hands during their period of recovery from the anesthetic effect of ice.

In each instance, an evaluation of the success of the operation was made following recovery from anesthesia. Sectioning of the facial nerve was expected to result in paralysis of some of the facial muscles

(Fig. 1). Most noticeably, the animals displayed a slackened jaw immediately after injury and immobility of vibrissae on the injured side. Furthermore, the eye on the experimental side could not close normally, so the eye-blink reflex could not be evoked following a successful operation. Since developmentally the vibrissae first move at 10 days of age and the eyes open at 15 days, some of these symptoms obviously could not be observed in the younger animals sacrificed at short postoperative times. The signs of facial nerve destruction appeared at the appropriate time developmentally in all long-term experimental animals. In all cases, the wound was later reopened at the time of sacrifice and the area around the stylomastoid foramen examined for the absence of the facial nerve. At no time was there evidence of reestablishment of peripheral connections; in fact, there was no axonal regrowth other than a tangle in the immediate area of the stylomastoid foramen.

Light microscopy: Histological procedures

After survival for the desired postoperative time period, the animals were anesthetized for perfusion fixation. A gravity-directed perfusion set-up allowed for the sequential flow of saline and formalin mixtures. The two perfusion fluids were warmed to room temperature and placed into i.v. drip bottles on a high shelf. The outflow from each bottle was directed to a common Y-type tubing, allowing for a change in type of fluid without interrupting the flow of perfusate. A cannula was placed on the lower end of the tubing that extended distal to the Y-tube, and the flow of perfusate was begun, to ensure that no

air was left in the system. Cannulas differed in diameter according to the size of the animal: they were all made by filing the bevel off of differently sized hypodermic needles.

The chest cavity of the animal was then opened, and a cut was made in the left ventricle of the heart to permit insertion of the cannula. The right atrium was incised as the flow of perfusate was begun. The cannula was advanced into the aorta and held there for the remainder of the perfusion.

A wash solution of saline and gum acacia (LaVelle and LaVelle, 1958b) was introduced into the animal first, followed by a mixture of saline, formalin and gum acacia (Koenig, Groat, and Windle, 1945). The purpose of the gum acacia is to govern the osmolarity of the perfusate, thus preventing cell shrinkage.

Following perfusion, the brains were exposed by removal of the cranium and further fixed *in situ* for at least 3 days in 10% formol-saline. This increased time of fixation *in situ* prevents the hyperchromatic response which occurs in neurons when perfusion is not complete or the brain is removed from the skull too soon (Cammermeyer, 1960; Friede, 1963; Ebels, 1975).

Following removal from the skull, that portion of the brain stem containing the facial motor nuclear group near the pontomedullary junction was dissected out for light microscopic study. Tissue was dehydrated in a graded series of alcohols, was infiltrated with several changes of Parlodion mixtures (2%, 4%, 6% in ethyl ether: absolute alcohol, v:v 1:1). For embedding, tissues were placed in a 6% Parlodion mixture in paraffin-lined Stender dishes. The dishes were

put into a dessicator containing chloroform vapors, and the blocks were hardened in the presence of these vapors. Blocks were subsequently removed, trimmed, and cleared in several changes of benzene (used in a hood). To complete the double-embedding methods of LaVelle and LaVelle (1958b), the blocks were infiltrated with paraffin (Fisher Tissue-Prep, 56.6° C) *in vacuo* for 30 minutes at 15-20 pounds of negative pressure. Blocks were hardened in cold water.

Blocks were sectioned at 5 microns on an A-O sliding microtome, using the 'wet blade' technique. Serial sections through the region of the facial nucleus were mounted on albuminized slides and left on a warming plate overnight. For cytological examination, slides were stained in buffered thionin (pH 4.46) according to the method of LaVelle (1951). Sections were mounted with Permount or HSR mounting medium.

Light microscopy: Cell counts

On random sections the approximate boundaries of the facial motor nucleus (FMN) were marked off with ink under the dissecting microscope. The FMN areas in three such sections, on each side of the medulla, were used for the cell counts for each animal. In experimental animals one side was ipsilateral to the axotomy, while the other side served as control. Both neurons and glia were counted in the FMN samples of all brains. The glia were identified according to their nuclear characteristics (Ling, *et al.*, 1973), and since only the nuclei stained with thionin, they were the units counted. For neurons, only those sections of cell bodies which included a prominent nucleolus were counted.

Counts were performed with the aid of a 1 cm square ocular grid used in conjunction with an oil immersion objective (100X); the outline of the grid could be seen over the cells of the facial motor nucleus. By means of a differential cell counter, all of the different types of cells could be counted at the same time in the same field. The cells in five grid areas per section of facial motor nucleus were counted, and this was the value recorded for purposes of statistical analysis. This procedure was repeated two more times per animal in each series, i.e., five grid areas in each of three sections of FMN were counted for each animal. As mentioned above, three animals were used for each series, so, in total, nine sections of normal and nine sections of experimental FMN were counted for each postoperative period.

Each 5-grid count was also considered to be characteristic of a unit of volume ($2 \times 10^5 \mu^3$) which was equal to the dimensions of the area counted times the thickness of the section itself. Such a unit of volume was thus shaped like an elongate, very thin box and was of the same size for all counts made. In both normal and experimental FMN, cell numbers of neurons and glia 'per count' were recorded for each animal age and at each postoperative time period, to be used for later analytical procedures.

Although considerable thought was given toward extrapolating counts to more standard volumes (e.g., 1 mm^3) or even to total cell populations, the effort was abandoned because of the lack of information concerning the somal volume of either the neurons or the glial cells. Furthermore, even if somal volumes had been estimated, there are no known correction factors that could be meaningfully applied to cells of

such variable size in a developing, shifting situation. (See Konigsmark, 1970, for existing correction factors for adult brain.) Only counts throughout serial sections or even throughout entire facial motor nuclei, could have realistically assessed the true number of glia and neurons, or the true ratio of glia to neurons within an extended volume of FMN. Since the aim of this study was to determine trends in population densities at many different ages and after several postoperative periods of time, the scope was too broad to include routine counting of serial sections. In the future, such a detailed analysis of selected, most reactive time periods might well be of interest.

The numbers obtained from the cell counts were subjected to statistical analysis in a variety of ways. Each of the values for each animal (the normal developmental series and the normal and experimental sides of the operative series), as well as the calculated averages for each cell type, were listed and coded for use in the FORTRAN system. A version of the statistical package for the social sciences (SPSS) program was chosen, such that the descriptive portions gave the mean, standard error and standard deviation for all values studied. Inferential statistics were then used to calculate the probability of significant differences in the populations. For this, two separate groups of data were examined. The sequential series of normal developing hamsters were examined to detail the differences between populations at different ages during normal development. The counts from the normal and operated sides of all of the experimental animals were compared to detail the differences within each set of animals (i.e., Ad-4 dpo normal side vs. Ad-4 dpo operated side. The contraction 'dpo'

stands for 'days postoperative.') The two-tailed T test was employed for testing the data from the experimental series. These 'side-to side' comparisons indicated the changes in density of cell populations at each age.

An additional series of data were analyzed to examine possible differences between the control developmental series and the normal sides of comparable experimental animals of the same ages. Any deviations in the normal side of operated animals from the normal control FMN of the same age would compromise the results on the experimental side. Briefly, all of the normal sides at 50 dpo were compared with the adult normal of the developmental series. Equally, all of the adult normal sides of the various dpo time periods were also compared with the completely normal values. All data from the control sides of animals in the experimental series whose chronological age came within 1 day of 5, 10, 15, or 20 days of age were compared with the data from the animals of the developmental series. This was an additional control to ensure that the operation, including relatively prolonged anesthesia did not affect the development of the animal or cause trauma to which the cells might respond.

Electron microscopy: Techniques and sampling procedures

On the basis of the results found with light microscopy, ultrastructural examination centered around two developmental stages: the 1-day-old and the adult hamster. Axotomy was performed in the same manner as previously described, and the postoperative time periods were 5 days and 100 days for the adult animals. A 3-day postoperative time

period was used for the 1-day-old axotomy series, for purposes of more closely monitoring this rapidly changing population of cells. In every case, at least four animals were used for each postoperative time period.

The preparation of tissue for electron microscopy was as follows: cardiac perfusion was performed on anesthetized animals in a 2-part procedure with first a weak (1% : 1.25%) glutaraldehyde : formaldehyde cacodylate buffered solution for 5-10 minutes (depending on the size of the animal) followed by a stronger (4% : 5%) concentration of the same solution, according to the method of Peters (1970). The glutaraldehyde (Fisher, Biological Grade) was purified by saturating with barium carbonate overnight and filtering the solution before use. The cacodylate buffer (0.2M) was corrected to pH 7.2 and the final solutions each had a pH of 7.3-7.4. The formaldehyde was prepared from para-formaldehyde and sodium hydroxide (Peters, 1970).

Following perfusion, the brain was exposed and the entire animal was sealed in a plastic bag and put in a refrigerator overnight. This provided the tissues with sufficient time to harden and protected the brain from distortion during the handling. A section of medulla and pons containing the facial motor nucleus was removed from each side, washed and postfixed in osmium tetroxide (1% in cacodylate buffer) for 1-2 hours. The tissue was further trimmed, washed, and dehydrated in a graded series of alcohols. It was then infiltrated with varying concentrations of propylene oxide and plastic (1:1, 1:3). The tissues were flat-embedded in a mixture of Epon 812 and Araldite 502 (according to Epon-Araldite Embedding Procedure recommended by LKB Instruments, Rockville, MD). Thick sections were cut on a Porter Bloom MT-1

microtome and stained with toluidine blue for orientation. After the facial motor nucleus was seen to be represented in the thick sections, the blocks were retrimmed and thin sections were cut on a Sorvall MT-2 microtome. Sections in the silver-gray interference color range were picked up on cleaned copper grids (200 mesh) and stained with uranyl acetate (saturated in 100% methanol) and Reynold's lead citrate. Sections were examined at 50 kV on an RCA EMU3F or at 75 kV on an Hitachi HU 11B2 microscope.

After locating the FMN in the blocks, at least 20 thin sections were collected and stained. After this, several more thick sections were taken and at least 20 more thin sections collected. This insured a sampling of different areas in the nucleus. This sampling procedure was repeated three times per animal. Every glial cell showing a nucleus was photographed. For the adult series this involved viewing well over 500 cells and for the 1-day-old series over 400 cells.

Photographic procedures

Photography of the light microscopic sections (both double - embedded material and thick sections of plastic material) was performed on a Zeiss Luminars Model microscope with Kodak Pan-X (ASA 32) film. An additional contrast filter, golden amber, was used for this procedure. Photography of the electron microscopic images was performed on acetate film Kodak #4489.

Miscellaneous additional procedures

For the light microscopic investigation of normal maturational patterns, several normal animals were used. These were never operated

upon and were examined for the changes seen in overall maturation. Several animals at various postnatal ages were chosen for light microscopy as follows: 1, 5, 10, 15, and 20 days old and adult ages. The tissue was collected and prepared in the same manner as for the experimental animals. Cell counts were performed in the same way, using 2 animals per age group and counting cells in the FMN on both sides of the brain stem.

For electron microscopy, several normal animals of pertinent ages were selected for study. Adult, 1 day and 4 day old animals were used and served as control for the normal developmental changes seen in this ultrastructural study. Many more animals were used to learn every aspect of the ultrastructural techniques. Typically, many very young animals were prepared for examination to ensure that at least 4 were well fixed.

As a comparative measure, facial nerve axotomy was performed on the right facial nerve of adult mice. The mice were of the CB57 strain. All operative and histological procedures were performed in the same manner as for the hamster series. The tissue was examined with the light microscope for nucleolar and cytoplasmic detail, although no cell counts were performed.

RESULTS

Morphological observations: Light microscopy

In the newborn, as well as adult, hamster the facial motor nucleus (FMN) is located in a ventrolateral position in the medulla (Fig. 2). Despite differences in size and shape, the neurons of both the adult and newborn animals form a distinct nuclear group (Fig. 3). The sequential examination of maturing neurons as they undergo changes in size, shape and position (spacing, density) has been well documented (LaVelle and LaVelle, 1958a, 1958b, 1975) and, therefore, forms the basis for the present study of possible glial responses associated with known neuronal changes during maturation and after injury.

Before analyzing cell populations within the FMN with light microscopy, criteria for identification of the glial cells of hamsters were established. Normal adult animals were used for this purpose, since their cells could best be compared with descriptions in the literature for other species (Ling, et al., 1973). When stained with thionin, only the nuclei of glial cells could routinely be observed. They were generally rounded in shape, all of about the same size, and much smaller than the nuclei of the neurons (Fig. 3A).

Based on their morphological appearance, the glial cells were easily separated into two types (Fig. 4). Some cells exhibited the many dense clumps of nuclear chromatin which are typical of oligodendrocytes. Others had a single, usually centrally located, chromatin clump and a very scant amount of staining around the nuclear membrane.

These cells were astrocytes. There was no cell in the normal adult hamster that fit the criteria for the microglial cell.

Examination of other areas of the brain served as a check on the identification of glial types. Myelinated areas, such as the corpus callosum and the genu of the facial nerve, were examined for their predominant cell type, which was relatively certain to be the oligodendrocyte. It was found that the principal cell in myelinated areas did fit the description of the oligodendrocyte.

With the identification of astrocytes and oligodendrocytes quite firmly established for the adult, examination of the younger stages of the hamster brain then followed. All sections used for identification were of normal animals of the following ages: 20, 15, 10, 5, and 1 day of age and newborn (Figs. 5 and 6). At each of these ages cells with the characteristics of astrocytes and oligodendrocytes could be identified, but the astrocytes were very infrequently encountered in the newborn through to the 5 day old samples. Generally speaking, the glial nuclear configurations did not undergo any morphological changes during the period of postnatal growth.

The glia were next observed for possible reactions to the dramatic changes known to occur in the neighboring neurons as a result of axotomy (LaVelle and LaVelle, 1958b, 1959, 1975). Sections of brain stems were taken at various postoperative time periods after axotomy performed at different ages and were examined in order to note the morphology of the glial cells.

After axotomy of the facial nerve in the adult, neurons of the motor nucleus became chromatolytic within five days (Fig. 7). This

chromatolytic appearance was gone by twenty days following axotomy (Fig. 8), and in that short span of time the neurons recovered their normal appearance. Throughout the adult postoperative series the glial cells showed no morphological change (Figs. 7 and 8).

Axotomy of 20-day-old animals elicited results similar to those in the adult. As expected, the neurons underwent an adult type of chromatolysis (Fig. 9), which lasted slightly longer than in the adult. The glial morphology did not exhibit changes at any of the postoperative periods studied.

After operation of both 15-day-old and 10-day-old hamsters, the neurons exhibited a different type of chromatolysis, more focal in nature than in the adult (see Literature Review). There was little if any swelling (Fig. 10 and 11) and a normal Nissl pattern reappeared by 20 days following injury. Occasionally, the chromatolytic response followed a more typically adult pattern. The glial morphology (Figs. 10 and 11) remained unaltered in all instances.

In the two youngest experimental series, definite degenerative changes took place in the neurons, resulting in considerable neuronal death. Yet there appeared to be no change in the glial morphology. By 5 days after axotomy of the 5-day-old hamster, the neurons were markedly chromatolytic and showed signs of regression (Fig. 12). A gradual decrease in the number of remaining neurons per sampled volume was apparent at succeeding postoperative ages. By 100 days post-operative only a glial scar remained (Fig. 13). Axotomy at birth resulted in neuronal cell loss as early as 10 days postoperative (Fig. 14). The resulting glial scar (Fig. 15B) seen 50 days following

the injury appeared similar to the glial scar seen in the 5-day axotomy series (Fig. 13B). These cells forming the glial scar could be classified as typical astrocytes and oligodendrocytes, and they showed no apparent change in nuclear morphology at any of the postoperative periods studied at each age.

A portion of this project involved a comparative study of the response of adult mice to axotomy. The mouse was chosen because of the extensive documentation of its striking neuronal loss after injury (see Literature Review). In the present study, neuronal loss in the facial nucleus of the mouse was detectable as early as 30 days following axotomy in the adult (Fig. 16). In addition, the nerve cells showed a definite chromatolysis (Fig. 17) long after hamster neurons had regained their normal Nissl pattern. The glial cell types were similar to those seen in the hamster, and here, too, there seemed to be no overall change in morphology of these cells following axotomy, at least as viewed with light microscopy.

Differential cell counts: Light microscopy

Cell counts were first performed on the facial motor nucleus (FMN) for the purpose of determining the numerical relationships of neurons and glia during normal development. The cells routinely counted were neurons, astrocytes, and oligodendrocytes. These figures subsequently provided a baseline for interpretations of the changing cell populations in the experimental portions of this project.

The counts showed (Fig. 18) that the numbers of neurons per sampled volume ($2X10^5\mu^3$; see Materials and Methods) of the FMN decreased

with age, while the number of astrocytes increased and the number of oligodendrocytes increased sharply to a peak value at 5 days of age and then fell gradually back to the perinatal level, which was retained in the adult. This adult value was reached at about 20 days of age. A closer examination (Table 1) of the neuronal numerical values per sampled volume revealed a large population of closely packed cells in the 1 day old hamster (64.0± 1.0), which became rapidly dispersed by 5 days of age (46.5± 3.5) and continued this rapid dispersal (10 days = 29.0± 1.0; 15 days = 22.5± 1.5) till the animal was 20 days of age (18.5± 1.5), when an adult level was reached (19.0± 1.0).

The density of oligodendroglial population (Table 1) was numerically equal to the neuronal at 1 day of age (66.5± 4.5), but underwent a dramatic increase by 5 days of age (116.0± 3.0). This peak value dropped slowly throughout development (10 days = 109.0± 2.0; 15 days = 90.5± 1.5) until 20 days (62.5± 3.5), when it reached adult levels (68.5± 1.5). The astrocytic population was very small (1.0± 0.0 per sampled volume) for the first five days (Table 1) but rose slowly throughout subsequent development (10 days = 6.0± 0.0; 15 days = 9.5± 0.5; 20 days = 13.0± 1.0) to adult levels (18.0± 0.0), which were considerably less than the values for the oligodendrocytic populations (68.5± 1.5) but approximately 1:1 numerically with the neuronal population.

When these age-dependent trends within each cell population were viewed comparatively (Fig. 18), certain correlations could be made. At the time of the earliest decrease in neuronal density (5 days of age), the density of the astrocytic population had not changed, yet the

Table 1: Number of cells per sampled volume ($2 \times 10^5 \mu^3$) in the developing FMN of the hamster. Numerical values indicate the mean ± two standard errors.

AGE	NEURONS	OLIGODENDROCYTES	ASTROCYTES
1 day	64.0 ± 1.0	66.5 ± 4.5	1.0 ± 0.0
5 day	46.5 ± 3.5	116.0 ± 3.0	1.0 ± 0.0
10 day	29.0 ± 1.0	109.0 ± 2.0	6.0 ± 0.0
15 day	22.5 ± 1.5	90.5 ± 1.5	9.5 ± 0.5
20 day	18.5 ± 1.5	62.5 ± 3.5	13.0 ± 1.0
Adult	19.0 ± 1.0	68.5 ± 1.5	18.0 ± 0.0

oligodendrocytic population had reached its peak value. Both the neuronal and oligodendrocytic populations reached adult values by 20 days of age, while the astrocytic numbers were still increasing and were significantly different at 20 days from the adult ($p<0.01$).

Once the normal developmental shifts in populations had been ascertained, differential counts were also performed on the FMN of animals in which the facial nerve had been severed at various postnatal ages, as stated in Materials and Methods. The purpose was to show any changes in density of glial populations which might accompany the neuronal responses to axotomy. It should be noted that the values for the normal side of operated animals showed no significant differences from those values of wholly normal animals of the same age.

Axotomy at 1 day of age resulted in major changes in all cell populations examined (Fig. 19). Comparing operated and control sides of the animals as early as 5 days postoperative (dpo), the number of neurons per sampled volume dropped significantly ($p<0.001$, Table 5) from 49.7$\pm$ 1.2 to 3.3$\pm$ 0.3 (Table 2). This was a much more precipitous drop than occurred normally with age (see Fig. 19 and Table 2). This significant decrease in number of neurons was present at all post-operative ages examined (Table 5). By contrast, the oligodendroglial population (Table 3), although increased in density after axotomy, was not significantly different from the normally increased population until the 20-day postoperative time period ($p< 0.005$, Table 5), when the reactive oligodendrocytes remained high in count and almost double in density of the control side (experimental = 122.7$\pm$ 3.5; normal = 65.7$\pm$ 0.7). The high value for oligodendrocytes on the operated side

Table 2: Number of neurons per sampled volume ($2 \times 10^5 \mu^3$) in the developing FMN of normal and experimental hamsters. Numerical values indicate the mean ± two standard errors. N = normal side; OP = operated; dpo = days postoperative.

AGE	DAYS POSTOPERATIVE							
	5 dpo		10 dpo		20 dpo		50 dpo	
	N	OP	N	OP	N	OP	N	OP
1 day	49.7±1.2	3.3±0.3	28.7±2.0	2.7±0.7	22.7±0.9	0.7±0.3	18.3±0.7	2.3±0.9
5 day	30.7±0.9	28.7±1.2	28.7±0.9	22.0±1.0	20.7±0.3	15.3±0.3	18.3±0.9	9.7±1.5
10 day	23.0±1.5	24.7±0.3	25.3±0.7	21.0±0.0	20.0±1.0	17.3±0.3	19.0±0.6	15.3±0.3
15 day	21.3±0.9	21.3±1.5	19.3±0.7	19.7±1.5	18.3±0.3	22.0±1.0	19.7±0.7	17.7±0.7
20 day	17.3±0.7	18.7±0.3	20.0±0.6	20.7±0.3	20.0±1.5	20.0±1.5	19.0±0.6	19.0±0.6
Adult	18.7±1.2	17.7±0.9	18.7±1.2	21.7±0.9	22.0±0.0	19.0±1.5	19.0±1.0	20.3±0.7

Table 3: Number of oligodendrocytes per sampled volume ($2 \times 10^5\ \mu^3$) in the developing FMN of normal and experimental hamsters. Numerical values indicate the mean ± two standard errors. N = normal side; OP = operated; dpo = days postoperative.

AGE	DAYS POSTOPERATIVE							
	5 dpo		10 dpo		20 dpo		50 dpo	
	N	OP	N	OP	N	OP	N	OP
1 day	115.7±3.4	127.0±4.5	105.7±2.6	120.0±4.0	65.7±0.7	122.7±3.5	70.7±4.7	93.0±3.5
5 day	109.3±1.5	109.0±1.2	95.3±1.5	109.7±4.2	67.7±0.9	77.0±4.2	65.0±1.0	60.3±2.7
10 day	77.7±1.9	73.3±0.9	69.7±1.8	67.7±2.2	76.3±2.0	71.3±1.8	71.3±0.7	67.3±0.9
15 day	69.3±2.0	69.0±3.1	67.0±1.2	64.7±1.7	74.0±6.2	73.7±5.4	68.7±3.0	65.0±1.5
20 day	69.0±1.2	63.3±1.3	68.3±1.2	63.3±1.2	67.7±2.2	69.7±1.7	64.7±0.9	69.0±0.6
Adult	63.0±5.3	64.3±6.1	62.0±3.6	71.0±2.1	69.0±2.0	66.7±1.8	66.0±3.0	71.3±0.3

Table 4: Number of astrocytes per sampled volume ($2 \times 10^5 \mu^3$) in the developing FMN of normal and experimental hamsters. Numerical values indicate the mean ± two standard errors. N = normal side; OP = operated; dpo = days postoperative.

AGE	DAYS POSTOPERATIVE							
	5 dpo		10 dpo		20 dpo		50 dpo	
	N	OP	N	OP	N	OP	N	OP
1 day	1.7±0.3	27.0±2.9	4.3±0.3	23.3±1.2	19.3±0.3	32.3±0.3	19.7±0.9	34.0±1.2
5 day	5.3±0.3	9.3±0.7	11.7±0.9	30.3±0.9	16.3±1.2	23.3±0.7	17.7±0.3	15.0±1.2
10 day	6.0±0.0	16.7±0.9	10.0±0.0	21.0±0.6	12.7±0.7	12.7±1.2	15.7±1.2	17.3±1.5
15 day	13.7±1.8	16.3±2.9	14.3±0.3	15.3±0.3	15.7±0.3	18.0±2.0	16.0±2.1	16.3±1.2
20 day	17.7±0.9	17.7±1.5	18.0±1.0	17.0±1.0	18.3±1.9	17.0±1.2	16.3±0.9	18.3±0.3
Adult	14.7±0.9	16.0±1.5	17.7±0.3	17.7±0.7	19.0±1.7	18.7±3.3	12.0±2.1	15.0±1.0

Table 5: The p values obtained from a statistical analysis of data in Tables 2, 3, and 4, comparing normal and experimental FMNs within each operated group.
AGE = age at operation; DPO = days postoperative.

AGE - DPO	NEURONS	OLIGODENDROCYTES	ASTROCYTES
1- 5	0.001	0.287	0.010
1-10	0.003	0.136	0.004
1-20	0.001	0.005	0.002
1-50	0.004	0.045	0.001
5- 5	0.184	0.423	0.057
5-10	0.070	0.040	0.008
5-20	0.004	0.115	0.044
5-50	0.006	0.215	0.094
10- 5	0.300	0.166	0.007
10-10	0.479	0.074	0.003
10-20	0.094	0.291	1.000
10-50	0.053	0.020	0.199
15- 5	1.000	0.950	0.383
15-10	0.860	0.369	0.225
15-20	0.053	0.742	0.296
15-50	0.075	0.487	0.742
20- 5	0.270	0.014	1.000
20-10	0.529	0.138	0.622
20-20	1.000	0.074	0.686
20-50	1.000	0.023	0.074
Ad- 5	0.478	0.578	0.270
Ad-10	0.350	0.165	1.000
Ad-20	0.188	0.222	0.860
Ad-50	0.456	0.251	0.188

was still significant at the 50 dpo time period also ($p<0.045$, Table 5). Increases in the astrocytic population were such that values (Table 4) showed significant differences (Table 5) at all postoperative times examined. As early as 5 dpo the number of astrocytes on the experimental side was 27.0± 2.9 per sample volume as compared to the normal value of 1.7± 0.3.

The graph of cell populations following axotomy at 1 day of age (Fig. 19) summarizes the trends seen. The neurons almost totally disappeared as early as 5 days after axotomy, while the density of the astrocyte population rapidly increased during that same period and remained significantly different from the normal throughout the entire postoperative sequence. Although the population density of oligodendrocytes on the experimental side was always greater than in the normal, these values did not reach significant proportions until 20 dpo, when the residual high count on the operated side far surpassed the normally declining values at that age.

Axotomy at 5 days of age elicited changes in the density of each of the cell populations examined (Fig. 20), though not so markedly as at day 1. The number of neurons per sampled volume was routinely less on the experimental side than on the control at all postoperative periods (Table 2), becoming significant by 20 dpo (Table 5). By 50 dpo, the neuronal density had decreased to 9.7± 1.5, as compared to the normal value of 18.3± 0.9 ($p<0.006$, Table 5). The oligodendrocytic change was less clear-cut. There was an apparent delay in the normal population decline, such that at 10 dpo the number of reactive oligodendrocytes per sampled volume (Table 3) remained at 109.7± 4.2, as

compared to the normal value of 95.3± 1.5 ($p<0.04$, Table 5). However, by 50 dpo the density of oligodendrocytes on the experimental side was similar to the normal value (experimental = 60.3± 2.7; normal = 65.0± 1.0) and was no longer significantly different. The astrocytic population was significantly changed throughout most of the postoperative period. By 10 dpo the density of astrocytes (Table 4) reached a peak value of 30.3± 0.9 as compared to the normal of 11.7± 0.9 ($p<0.008$, Table 5). However, as with the oligodendrocytes, by 50 dpo the number of astrocytes per sampled volume was similar for both control and experimental groups (normal = 15.0± 1.2; experimental = 17.7± 0.3).

To summarize these population trends (Fig. 20), the neurons diminished in number slowly, to eventually half of the normal population. The astrocytes increased markedly but transiently early in the postoperative period. The oligodendrocytes followed normal values more closely, showing a temporary delay in the normal decline in population density. This delay coincided with the most striking increase in astrocyte density (at 15 days of age; 10 days postoperative). By 50 dpo the population densities of both types of glial cells were essentially normal in value.

Axotomy at 10 days of age resulted in few changes in cell populations (Fig. 21). The change in neuronal population (Table 2) followed normal values quite closely until 50 dpo, when there was a slightly greater decrease ($p<0.05$, Table 5) in the density of neurons on the experimental side (15.3± 0.3) as compared to the normal (19.0± 0.6). The density of the oligodendrocyte population remained essentially constant throughout the postoperative period (Table 3), with values

approximating 70. The population density of astrocytes increased initially (at 5 and 10 dpo) but was normal in value by 20 dpo (Table 4). At 5 dpo the astrocytes on the experimental side numbered 16.7± 0.9 per sampled volume as compared to the normal 6.0± 0.0 ($p < 0.007$, Table 5). The values at 10 dpo were still significantly different (experimental = 21.0±0.6; normal = 10.0±0.0, $p < 0.003$). The changes seen in this experimental series (Fig. 21) were mainly in the astrocytic population. The density of astrocytes increased soon after the axotomy. However, by 20 dpo the value for the experimental side (12.7± 1.2) was nearly identical to the normal (12.7± 0.7), and the astrocytic populations on both sides underwent the normal increase thereafter, so that values at 50 dpo were still similar (experimental = 17.3± 1.5; normal = 15.7± 1.2).

Differential counts of cell populations following axotomy at 15 days of age and older showed no significant changes from the normal for any cell populations (Table 5). Cell counts per sampled volume for each postoperative period are shown in Tables 2 (neurons), 3 (oligodendrocytes) and 4 (astrocytes). Similarly, the graphed comparisons for each cell population are shown in Figures 22 (operated at 15 days), 23 (operated at 20 days), and 24 (operated as adults).

Morphological observations: Electron microscopy

As already described, differential cell counts reflected distinct age-dependent types of response to axotomy, ranging from neuronal death and glial proliferation at early ages to no detectable changes in either population (beyond a transient neuronal chromatolysis) in the older animals. The two extremes of response (after axotomy at 1 day of age

and in the adult) were chosen for further examination ultrastructurally. The purposes of this portion of the study were (1) to verify the identification of the glial elements present in both the normal and experimental facial motor nuclei, and (2) to seek evidence of the phagocytic activity which presumably accompanied the disappearance of neurons after axotomy at 1 day of age.

In general, the thick plastic sections stained with toluidine blue revealed the compact architecture of the FMN (Fig. 25), with large motor neurons separated by a 'neuropil' which contained neuronal processes and the nuclei of identifiable oligodendrocytes and astrocytes. In many cases neuronal somas lay next to blood vessels (Fig. 25). Also, small, discrete, organized bundles of myelinated fibers could be found running through the area, with their accompanying oligodendrocytes.

In the normal adult series, thick sections showed both typical large motoneurons and smaller nerve cells of normal appearance. Neuronal characteristics (Fig. 26A) included (1) a prominent nucleolus within a vesicular nucleus and (2) cytoplasm containing large clumps of lipofuscin and basophilic material (Nissl substance). Also visible were discrete spots in the nucleus which were seen as aggregates of granular pink-staining material. Occasional deep invaginations in the nuclear membrane could also be seen in the normal facial motor neurons. (These nuclear features remained after axotomy and are illustrated in Fig. 28).

The criteria for identification of glial cell nuclei in thick Epon sections were the same as for the double-embedded preparations viewed by light microscopy. Morphological characteristics were identical in normal and operated FMN's. The astrocyte nucleus had one large

chromatin body (Fig. 27A), while the oligodendrocyte had several large coarse clumps (Fig. 27). The nuclei of oligodendrocytes could often be found in a perineuronal (satellite) position (Fig. 27 B, C), as well as lying isolated in the neuropil, quite separate from the neuronal soma. The nuclei of astrocytes were scattered throughout the neuropil.

When viewed with the electron microscope, the adult facial motor neurons were quite characteristic (Fig. 29). Their cytoplasm contained all common organelles, with an abundance of protein synthetic machinery. The stacks of rough endoplasmic reticulum (RER) and Golgi were quite striking in the mature cell. The nuclear envelope was round to oval and typically showed few indentations of its membrane. The nucleus was very large, generally devoid of chromatin clumps, and marked by the presence of a large, spheroid nucleolus which lay free in the nucleus, not touching the nuclear envelope. The granular and fibrillar components of the nucleolus were easily seen, along with some perinucleolar chromatin.

An inspection of the appositions on the neuronal soma and dendrites revealed many synaptic contacts as well as non-specialized glial contacts. In some cases oligodendrocytic nuclei were seen in an immediate perineuronal position, their cytoplasmic membranes in direct contact with the neuronal cell membrane (Figs. 30 and 31).

The term 'oligodendrocyte' is a name given to a family of cells varying in size and in staining density. The cells seen in this study were typical of those already described in the literature. Their general configuration included a large, ovoid, eccentrically placed nucleus, surrounded by considerable cytoplasm. Their chromatin was

arranged in clumps in the center of the nucleus, as well as next to the nuclear envelope (Figs. 30 and 32). The cytoplasm contained many microtubules and cisterns of RER (Figs. 30 and 33). In the adult hamster it was possible to observe differences in the stainability of the oligodendrocytes, but only light and dark types could be identified (Figs. 32 and 33). The cells diminished in size as their staining intensity increased.

The appearance of the astrocytes of the hamster followed closely the classical descriptions for this cell. They exhibited a very light staining nucleus and cytoplasm (Fig. 34A). The nuclear envelope was beaded with small chromatin clumps, and a single dense spheroid of chromatin was usually seen in the nucleus, occasionally lying free from the edge of the nuclear envelope. The pale cytoplasm resembled that of axons and characteristically contained prominent bundles of filaments (Figs. 34B and 35) which extended out into the long slender processes. Astrocytic processes were unusually complex and pervasive throughout the neuropil.

As seen in thick plastic sections, the changes observed four days following axotomy in the adult hamster involved only the motor neurons, which became typically pale and swollen (Fig. 26B). Nuclear characteristics were unchanged from the normal (Fig. 28). Although the course of cytoplasmic recovery was not followed step by step in this series of experiments, a facial nucleus observed 100 days after axotomy revealed normal neurons with darkly staining Nissl substance. There did not seem to be any remarkable changes in the glial cells at either 4 or 100 days following axotomy nor was the neuropil remarkable at either of the

postoperative ages.

On the ultrastructural level chromatolysis most conspicuously involved changes in the RER of the neurons. Normal Nissl bodies were seen as aggregates of RER with parallel cisterns and many free ribosomal rosettes between them (Fig. 29). Following axotomy, however, these cisterns dispersed and broke into short segments (Fig. 36). The ribosomes became scattered throughout the cytoplasm. The adult neurons showed no dramatic increase in lysosomal structures or autophagic vacuoles following axotomy. The neurons of the adult-100 dpo hamsters showed an increase in aggregates of RER as they recovered their normal cytoplasmic appearance (Fig. 37).

The glial cells revealed no morphological changes during the neuronal response to injury in the adult. Both oligodendrocytes and astrocytes were readily identified. There was no increase in lysosomal structures in either of these cell types.

Of over 500 separate cells examined, no cell remotely fit the criteria for the classical microglial cell (see Literature Review). Special attention was given to cells surrounding blood vessels, but these were all observed to be non-microglial. One cell resembled a mast cell.

Cellular relationships were somewhat different in the series of young hamsters axotomized at 1 day of age and examined 3 days later. The unoperated side served as a control for cell types, and it should be noted that in appearance it did not differ from the FMN of the normal, unoperated 4 day old animal. Thick plastic sections of the normal 4-day old FMN revealed the presence of a group of closely packed

neurons. The cells were large, with a prominent nucleus and sometimes multiple nucleoli (Fig. 38A). Glial nuclei were much smaller (Fig. 38) and almost entirely oligodendrocytic in type with only an occasional astrocytic nucleus present. The remainder of the cells in these sections were endothelial or closely associated with blood vessels. Axons were as yet not myelinated, so, as a whole, these sections of FMN seemed to be rather evenly stained.

Upon ultrastructural examination, the neurons at 4 days of age were typical of descriptions in the literature for such immature cells. Their nuclei were prominent and typically showed infoldings of the nuclear membrane (Figs. 39, 40, and 41). There were some well formed aggregates of RER in the cytoplasm of most neurons (Figs. 39 and 41).

The identification of glial cells was problematical at this young age. Although their nuclear configuration usually was identifiable as oligodendrocytic or astrocytic, many cells could also be classified as oligodendroblasts or astroblasts because of the immaturity of their cytoplasmic features. These cells were probably intermediate in their development toward mature glial types.

Many of the glial cells at this age had large stubby processes, unlike the thin pervasive types seen in the adult neuropil. Since the neuronal processes within the neuropil were small and generally unmyelinated, and since there was considerable extracellular space in the neuropil, it seemed possible that the 'packing pressure' of the neuropil had not yet compressed the glial processes.

It was necessary to examine many grids at this age before finding even a single identifiable cell of the astrocytic line. These cells had

none of the aggregates of cytoplasmic filaments seen in the mature cell. Only their nuclei indicated that they were astrocytic (Figs. 42 and 43).

Cells with large coarse clumps of nuclear chromatin (Figs. 42 and 43) typical of adult oligodendrocytes were readily found. Some of these oligodendroblasts (Fig. 42) had already begun to show stacked RER in their cytoplasm.

Again, no cells resembling microglia were identified.

As early as 3 days following axotomy at 1 day of age, the experimental FMN exhibited major changes from the normal. As seen in thick sections, the neurons were pale, with eccentric nuclei, and often exhibited features typical of dying cells (Fig. 44). These dying cells showed a very darkly staining nucleus surrounded by very little stainable cytoplasm. Within the nucleus the nucleoli were extremely pycnotic and bead-like in appearance. Also noted were very darkly staining clumps of debris (Fig. 45) usually found near glial cells.

Ultrastructurally, one could follow the rapid progressive breakdown of the neurons through various stages of degeneration. Some cells exhibited an overall dilution of cytoplasmic constituents and a noticeable disappearance of RER and free ribosomal material (Fig. 46). In the cytoplasm of other reactive neurons dense bodies and autophagic vacuoles could be found (Fig. 47). Inspection of the processes of these reactive neurons often showed a narrowing of the proximal part of the dendrites similar to descriptions in the literature of the 'pinching off' of processes by surrounding neuropil (Torvik, 1972) (Fig. 46). Dense neuronal debris of a granular condensed appearance (Fig. 48) was seen in abundance throughout the FMN area.

In scanning through the grids, one could detect the more numerous glial populations. Astrocytic cells did not show any morphological changes. Many oligodendrocytes and oligodendroblasts were seen near degenerating neurons (Fig. 49). Some of these glial cells showed alterations in their cytoplasm, in that they now contained large dense bodies and lipid droplets (Fig. 50). Cells of this type were frequently found in contact with debris. While such cells were oligodendrocytic in appearance, they could also fit the current electron microscopic description of microglia.

Examination of the normal FMN of the 1 day old unoperated animal was performed to determine whether any of these younger cells were similar in appearance to the reactive cell types seen 3 days after axotomy. No such similarities could be found.

DISCUSSION

The purpose of this project was to examine the glial population of the hamster facial motor nucleus (FMN) during normal development as well as after severance of the facial nerve at selected stages of development. It was anticipated that the differing neuronal response to axotomy at various critical stages might elicit different types of glial response. An important part of this work involved the identification of the various elements of the glial population in the hamster.

The histology of the developing facial motor neuron of the hamster is well documented in the work of LaVelle and LaVelle (1958a, 1958b, 1959, 1975). They also described the chromatolytic response in neurons at various postnatal ages. Their work resulted in a detailing of critical stages in neuronal development which could be correlated with differing responses to axotomy performed at various postnatal times. This prior work on the hamster provided an important baseline for the present study, since the ages chosen here were representative of known stages of neuronal maturation. There were no guidelines available for the development of glial cells in the hamster.

This study showed, as expected, that the facial motor neurons in the newborn hamster were very closely packed and had already migrated to their ventral position within the brain stem near the pontomedullary junction. They increased in size very rapidly between birth and 5 days of age and already began to acquire, at that early age, an adult-like nuclear configuration and density of Nissl substance.

It is known, however, that these facial neurons are not physiologically mature until 20-25 days and that morphologically their processes continue to elongate and become more complex until 25 days or more of age (LaVelle and LaVelle, 1958a, 1958b, 1959, 1975).

With light microscopy there was no real problem in identifying oligodendrocytes and astrocytes, but no cells were found which could be identified as being microglia. The oligodendrocytes and astrocytes were characterized by their nuclear configurations (Ling, et al., 1973), which were identifiable at all the ages studied. Their cytoplasm presented some problem ultrastructurally between birth and 4 days of age, when the cells were still in the glioblast stage, without their full complement of organelles.

The presence or absence of a population of microglia in the normal central nervous system can perhaps be regarded as a species difference (Cammermeyer, 1970). Vaughn and Peters (1968) stated that there were no microglia in normal rat brain, but that instead there was a transformed type of cell, inherent in injured states only. This theory predominated until 1970, when Cammermeyer demonstrated the presence of microglia in germ-free rats.

It is interesting to note that microglia are present in great numbers in the cortices of rats which have pneumonia (Peters, Palay and Webster, 1976). Also, the most widely used animal for studies of microglia is the rabbit, which chronically suffers from respiratory infections (Peters, Palay, and Webster, 1976). These authors suggest that a history of previous infection may change the populations of microglia within a given animal. Considering that these cells are a

part of the phagocytic system of the body, such an idea seems well taken. Perhaps the status of the immune system of the animal is a major factor in the number of microglia present. The immunocompetence of the adult hamster may, indeed, be of a different order from that of some other animals.

There was a time when only a few laboratories devoted their attention to the neuroglia, yet now these cells are being studied widely, not only for their origin and morphology, but for their biochemical and physiological properties as well. The possibilities for metabolic interrelationships with neurons have always existed, yet the precise populations of cells which may be interreacting are not known. Our aim, therefore, in making differential cell counts in the hamster FMN was to examine any fluctuations in the density of the glial population which might be correlated with neuronal developmental changes or with neuronal reactivity to injury. To date, no other study has followed sequential changes in the glial cells as related to injury of developing neurons.

The best means of expressing cell density in this study has been particularly difficult to determine. The differential counts were designed to enumerate the types of cells found in an actual volume of FMN (in this instance, $2X10^5\mu^3$ as described in Materials and Methods). In other words, the counts were an expression of cell density within a specific unit of volume. This sampled volume was never changed throughout all the age groups examined, although the total size of the FMN obviously increased with age. Because we are dealing with different ages, each of which has a different final facial nuclear volume of

undetermined extent, any factor for increasing the volume of the counts can not be an absolute or constant number. To establish a series of factors, for each age, would be a separate study in itself. Both the volume of the cell soma, which is highly irregular in shape, and the volume of the overall facial motor nuclear area would have to be assessed for each developmental stage. The absolute dimensions of the FMN would be particularly difficult to establish with certainty. The nuclear group does not have sharply defined boundaries along its circumference; it is not encircled by a nerve or tract. It is true that the rostral-caudal extent could be estimated by the appearance and disappearance respectively of the large motor neurons. However, that does not take into consideration any bordering glial elements nor any peripheral small neurons which may actually belong with the nuclear group rather than with the surrounding reticular formation. The counts per unit of constant volume ($2X10^5\mu^3$) were, therefore, designed to be relative numbers which could be directly comparable in and of themselves. Trends and fluctuations in populations can be expressed, but the total number of glia as related to the total number of neurons, in the FMN at any age will have to await the derivation of morphometric formulas and conversion factors which are not yet available.

The differential counts performed on the normal developmental series showed rather definite maturational trends. There was a continuous decrease in the number of neurons per sampled volume from birth until 20 days of age. Presumably this does not represent a continued net loss of total neurons but, rather, a spreading out of the population within an increasingly large volume of facial nucleus, as cell somas

grow in size and processes branch and lengthen. Such a spreading would be expected, of course, as evidenced from prior work detailing the events leading to an increase in neuropil and a decrease in population density, as measured by the distances between cell nuclei (Eayrs and Goodhead, 1959; Brizzee, et al., 1964; Ptacek and Fagan-Dubin, 1974). The neuropil becomes increasingly filled with processes and myelin as the neurons grow. As a result of this cellular growth, the brain itself increases in volume. So the decrease in neuronal numbers per unit of volume is a normal developmental trend and is a function of overall brain growth and the dispersion of a relatively stable population of cells. One other factor which may contribute to the decline in the population density of the neurons is the continued loss of neurons, which characteristically accompanies early development.

The population trends among glia during normal development of the hamster as revealed in this study followed quite different curves. The oligodendrocytes showed the greatest fluctuations in population density throughout development. In the newborn these were the most numerous elements (66 cells per sampled volume) in the FMN. Their numbers grew to a peak value (116 cells per sampled volume) at 5 days postnatal age. At 10 days postnatal age the value was still high, but thereafter the cell density gradually diminished to adult levels (68 cells per sampled volume). The decrease in the population density of oligodendrocytes during the later phases of normal development may represent a now stabilized glial population dispersing throughout an increasing volume of growing FMN. The timing of the massive early increase in oligodendrocytes immediately precedes the period of most

active myelination, which has been shown to begin at the seventh day postnatal age in the hamster brain stem (LaVelle, 1963). In other species (Vaughn, 1969) an increase in the oligodendroglial population is usually noted several days prior to the onset of myelination. The hamster seems to follow a similar sequence.

The density of the astrocytic population in the hamster FMN is very low at all ages, ranging from a mean of 1 cell per sampled volume in the newborn to only 19 cells in the adult. No large spurt in population density was observed at any particular age examined, but a continued increase through to the adult stage was seen. This continued increase throughout development is striking when one takes into account the increasing volume of the FMN. To maintain a continuing increase in density, in spite of the diluting factor of overall increase in FMN volume, proliferation of astrocytes must occur. It has been shown that astrocytes differentiate from cells leaving the subependymal layer in rats (Ling and Leblond, 1973) as late as 1 month of age. In a study on the ratios of glial cells present in different areas of the brain, Ling and Leblond (1973) reported an astrocyte population one-third the size of the oligodendroglial population. This is in agreement with the overall proportions of astrocytes and oligodendrocytes found in this study for the adult hamster.

When cell counts were performed to survey the density of populations during the neuronal response to axotomy, it was anticipated that there would be a proliferation of glia. Work on other species of animals has shown glial cells adjacent to the cell body of an axotomized neuron showing changes correlated with the neuronal response (Stenwig,

1972; Torvik, 1976; Chen, 1978).

The lack of any change in glial population density after axotomy in the older ages implies that a transient neuronal chromatolysis alone is insufficient to cause a proliferation of surrounding glia in the hamster. Only neuronal death, as observed at the youngest ages, is capable of eliciting this response, at least in the hamster. Neuronal death to some degree is, of course, a characteristic result of axotomy in most laboratory animals, even as adults.

The dramatic increase in glial population following axotomy at 1 day of age may result from a combination of factors. One cause, proliferation of cells, seems acceptable as mitotic figures were seen in the tissue prepared for ultrastructural analysis. Furthermore, the presence of a seemingly increased number of glial cells adjacent to profiles of injured neurons (prior to absence of neurons) indicates a proliferative activity. However, since no measure of this mitotic activity was undertaken, evidence for proliferation as the only cause of this increase is not available. One other factor may be the shrinkage of the total FMN as a result of neuronal loss, thus concentrating a given population of cells into a smaller volume. The ultimate decline in oligodendrocyte population density in the longest postoperative periods may be due, as already indicated for normal development, to dispersion within an overall increasing FMN volume. Only a detailed morphometric study will resolve this problem.

Our counts were designed to sample from a wide population of differently aged animals, after postoperative periods of varying length, so as to provide a range of numbers for analysis. It was intended that

the times of greatest glial response would later be selected for more detailed ultrastructural study. When only the youngest ages showed a dramatic glial response, it was decided simply to compare the ultrastructural responses at one day of age with those of the adult, which underwent no apparent changes after axotomy.

One of the objectives of the ultrastructural study of adult brain was to show whether any population of microglia could be detected at the electron microscopic level. Secondly, since the axotomized adult hamster neurons regain their normal appearance in a relatively short time after an initial chromatolysis, an ultrastructural profile of the reactive neurons would reveal the relative degree of ribosomal (chromatolytic) change typical of these neurons. Finally, the ultrastructural pictures would show glial/neuronal cellular appositions, and a survey of these would reveal any overt major contact changes such as have been described for cranial motor neurons in other species (Blinzinger and Kreutzberg, 1968; Sumner and Sutherland, 1973; Watson, 1974, Chen, 1978).

The results of the ultrastructural study of the adult brains confirmed the findings of the light microscopic examination. The initial neuronal changes resulting from a transitory period of chromatolysis were in agreement with those described in the literature (Torvik, 1972; Lieberman, 1974; Aldskogius and Arvidsson, 1978). While no morphometric quantitative analysis was undertaken, the ratio of glial and synaptic contacts was studied in some samples of normal and experimental FMN. No dramatic change such as that seen by Blinzinger and Kreutzberg (1968), was found.

The glial cells appeared quite normal and were easily identified in the FMN. Furthermore, neither the astrocytes nor the oligodendrocytes showed any 'reactive' changes in the experimental FMN. Again, the ultrastructural findings confirmed the absence of a population of microglial cells in normal or injured FMN.

Much work has involved the glial participation in synaptic displacement as seen ultrastructurally (Blinzinger and Kreutzberg, 1968; Sumner and Sutherland, 1973; Watson, 1974; Chen, 1978). Deafferentation of the injured cell may facilitate recovery to normal synthetic capacity as long-term studies show a return of synaptic contacts (Chen, 1978). Microglial cells are frequently implicated in removal of synapses. One area of controversy is the identification of the small processes or 'tongues' of cytoplasm as microglial processes. Chen (1978) has found that astrocytes as well as microglia temporarily displace synaptic contacts around injured neurons of adult cats. Furthermore, the cells identified by Blinzinger and Kreutzberg (1968) as microglia do not show the cytoplasmic characteristics of this cell type; they resemble oligodendroglial types.

Since axotomy shortly after birth results in rapid death of neurons, our ultrastructural study was intended to reveal details of degenerative and phagocytic processes taking place. Operations at 1 day of age were followed by sacrifice at 3 days postoperative (3 dpo), in the hope of 'catching' neurons at the height of their degenerative changes. Although no phagocytes had been identified in our hamsters at the light microscopic level, it seemed reasonable to expect that some type of debris uptake must occur. Whether active phagocytes might

be recognized ultrastructurally as being microglia was a possibility we entertained. Torvik (1972, 1975) has shown such to be true for adult mice and rats.

Our ultrastructural study detailed the process of neuronal disintegration in the experimental FMN of the young brains. The profiles of degenerating neurons agreed with those in the literature (Torvik, 1975; Aldskogius and Arvidsson, 1978). The speed with which the neurons disintegrated was related to the age of these cells at the time of injury. While other studies have employed the use of 'newborn' rabbits and rats, those animals both have a longer gestation period than the hamster and, therefore, are born more mature in a sense. From a comparative standpoint the present study would seem to be examining one of the youngest and, therefore, most vulnerable populations of neurons.

Ultrastructurally, the glial cells at early ages were problematical in their identification in both normal and experimental FMN. The glia were 'blast-like' in appearance but, again, the nuclear configurations proved to be the identification criteria. The glial cells were in abundance, as evidenced by the cell counts. While the number of astrocytes per sampled volume increased, they did not show any change in morphology at the early postoperative time period. The oligodendroglial cell types were usually found near profiles of cellular debris. One might expect that phagocytes would be near areas of debris, and these oligodendroglial cells showed morphological changes in their cytoplasm typical of a phagocytic cell. They contained many lysosomes, dense bodies and lipid droplets. This leads to the speculation that the

alteration in cytoplasmic morphology might indicate a different metabolism or function of that cell. Following this hypothesis, the conclusion that the oligodendroglial cells act as phagocytes in this situation seems plausible. Other workers have described a reactive type of oligodendrocyte following injury (Maxwell and Kruger, 1966; Hasan and Glees, 1972; Nathaniel and Nathaniel, 1977).

The profile of a 'reactive' oligodendrocyte closely resembles that of a characteristic 'reactive' microglial cell and that of the 'third type' of glial cell. Viewing the morphological similarities of oligodendrocytes and microglia, one is forced to consider the possibility that these cells are related. They may be intermediates, not in a developmental sense, but in terms of a transformation of cells to the reactive type seen. This interrelationship has been proposed in the literature (Carpenter, 1976).

Belief in the absence or presence of microglia as a distinct cell type is a result of interpretation of morphological criteria. A cell resembling the description of microglial cell types can be found in the reactive FMN near dying neurons of the young hamster only. The fact that no cell type could be found in the normal or reacting FMN of the adult hamster leads one to speculate that this cell seen only in areas of necrosis is a transformed oligodendrocyte performing a phagocytic function.

A factor that appears to result in differences in the retrograde response to axotomy is the age of the animal at the time of injury. Most types of young neurons are susceptible to axonal injury, and this has become an often used neuroanatomical technique for the localization

of cell bodies (Lieberman, 1974). These young neurons are more sensitive to all types of injury. It has been postulated that neurons of young mammals are deficient in a number of basic homeostatic mechanisms that form during the development of the animal (Lieberman, 1974; LaVelle and LaVelle, 1975). In comparing the results of many investigators, one must be careful about generalizing on the age at which axotomy invokes a differing response, since species differences often make direct age comparisons meaningless. The newborn rabbit, for instance, is more mature than newborn rats, which in turn are more mature than hamsters at birth. Only in studies such as the work of LaVelle and LaVelle (1958a, 1958b, 1959, 1975) and this project, which deal with specific ages within a single species, can one draw conclusions about differing responses based on neuronal age.

Another point for consideration in making comparisons is the meaning of the term 'young' or 'newborn.' In the present study 'newborn' means 12 hours or less old. However, some authors speak of using 'newborn' animals and later indicate their 'newborn' rabbits were 6-9 days old (Torvik, 1972) or that their 'newborn' rats were one week old (Torvik, 1975). This makes comparative interpretations of cellular responses very difficult.

Generalizations concerning the vulnerability of neurons cannot be made in accordance with the birthdays of animals. One must first examine the cytological indicators of maturity, in order to categorize the metabolic maturation of the neurons. This maturational state of the organelles of the neurons can then be used to predict the nature of the expected response following axotomy.

LaVelle and LaVelle have established morphological criteria of metabolic maturity in the hamster (1958a, 1975). The facial neurons at birth are still 'neuroblastic' in appearance, with multiple nucleoli and very little basophilic cytoplasm. The 'nucleoli' are as yet composed primarily of DNA (heterochromatin). By 5 days of age nucleoli have for the most part become single, and their RNA moiety is becoming prominent. At the same time, the neuron itself has increased markedly in size and displays prominent clumps of cytoplasmic Nissl substance. Neurons at 5 days are more resistant to injury than at birth. As the pattern of their nucleolar configuration becomes more adult-like, i.e., increased RNA with dispersion of DNA to the nucleolar periphery, they become increasingly competent to survive axotomy.

As shown by LaVelle and LaVelle (1975) in facial motor neurons of the hamster, the final cytological indication of the attaining of physiological maturation is the appearance of an RNA-rich intranucleolar body. This appears between 20-25 days of age, the time at which the neuronal response to axotomy becomes adult in character. The LaVelles equate this maturation with the attainment by the cell of the capability to synthesize and store sufficient protein for cytoplasmic repair. The younger the neuron, the lower is its capacity for regenerative response, and the more likely it is to die following axotomy. Any developing system is presumably directing its synthetic efforts toward maturation of organelles and the formation of new membranes. As suggested by the LaVelles (1975), when this synthetic machinery is called upon to repair and replace a large volume of axoplasm and membrane (as is the case resulting from severance of an axon), the cellular machinery may simply

not be at a status capable of coping with increased demands. On the other hand, if a cell is operating at a steady metabolic state, as is evidenced in the hamster by specific cytological indicators, it may have the capacity or reserves to meet the increased demands of repair and will, therefore, recover quickly from injury.

A comparison between the normal facial motor neurons of the adult hamster and those of the adult mouse is of interest here. The mouse motor neurons have much less Nissl substance accumulated in their cytoplasm, and the existing Nissl substance seems more flake-like. Also, while their single prominent nucleoli are advanced in their overall configuration, they contain no intranucleolar body (INB), such as would be typical of comparable facial neurons in the hamster. In other words, the mouse neurons present the cytological picture of 'not quite mature' cells. In a blind study made by colleagues in this laboratory involving photographs of adult cells, these normal mouse neurons were selected as 'immature' or 'chromatolytic' as measured against the standard of 'maturity' presented by hamster facial neurons. The fact that these mouse cells die in large numbers following axotomy lends support to the hypothesis that immature-appearing (i.e., less advanced) neurons are more vulnerable to axotomy. The fact that they are 'adult' neurons, and therefore as 'mature' as they can become, is beside the point in this instance. As shown by LaVelle and LaVelle (1958b), many adult neurons of the hamster are less advanced than facial neurons in their nucleolar configurations and their content of Nissl substance. It is assumed that they therefore have a lower capacity for protein production. At a time of injury and of consequent great demands

on synthetic capacity, these neurons, like those of the mouse, may not be able to cope. The 'species differences' in the response of mouse and hamster neurons to axotomy may rest, then, in their differing inherent capacities for 'coping with stress' by the production of proteins necessary for cell maintenance (Jacob and LaVelle, 1977).

The fact that facial neurons of the hamster seemingly recover so completely after injury raises interesting questions of another sort. As shown in the present study, whether 30 days or 100 days after axotomy performed at older ages, the experimental neurons appeared normal morphologically. This was true even though there was no functional return to the facial musculature. The absence of the eye-blink reflex on the operated side, as well as the continued lack of ipsilateral vibrissal movement, proves that the proper reconnection of the neurons with their effector organs had not been established.

It has been shown by other investigators, with both axotomy and crush lesions of peripheral nerves, that peripheral reconnection enhances the rate of recovery. Complete and rapid regeneration of the cell body, along with functional recovery, has been found following crush lesions in many species of animals. In mice (Torvik and Skjørten, 1971) there is an incomplete type of chromatolysis following crush lesions, while in rats (Torvik, 1976) crushing results in few light or electron microscopic changes. Rabbits (Torvik and Sõreide, 1975) and adult hamsters (Smith and LaVelle, personal communication) exhibit the classical chromatolytic reaction after crushing, rapidly followed by somal recovery and return to function. The survival of these neurons following crush lesion would seem to be directly related to the ease of

establishing their axonal reconnections.

For most species, marked cell death is reported following a cut lesion of the nerve, and the lack of functional reconnection is usually cited as the reason for cell death (Torvik and Skjørten, 1971b; Lieberman, 1974). In the hamster, however, cell death does not follow axotomy in older animals. The reasons for somal recovery are unclear. There is no doubt that, after long-term recovery, axonal sprouting takes place. Indeed, as seen in this study, the cut end of the facial nerve develops a typical neuroma, with a ball of tangled ends. Whether or not these ends establish ectopic connections with nearby muscle or connective tissue is as yet unknown. However, they do not reinnervate their original target mass. It would seem that, while original peripheral reconnection enhances the rate of neuronal regeneration, it is not necessary for the ultimate survival of these neurons in the adult hamster.

Speculations about the necessity for 'proper' reconnections raise a question concerning the nature of the signal for cytoplasmic recovery. It would seem obvious in the crush experiments that axonal regrowth through the connective tissue sheath would lead to the original target organ. The signal, though its exact nature is not known, might be that the axons are reinnervating a 'recognized' muscle. However, it has been shown that seemingly recovered neurons are later found not to have reestablished functional contacts. These cells frequently undergo a slow atrophy and disintegrate weeks later (Torvik, 1976).

For this present study the signal for chromatolysis, as well as the way in which the neurons react to it, forms the basis for the glial response. It would seem that the glial cells increase in number only as a result of extensive neuronal death. Considering the primary role of the glial cells as being 'supportive' in some way, even if in the form of metabolic interactions, one might presume that they would respond to neuronal injury under all circumstances, no matter what the outcome for the neurons. The glial cells present during the adult chromatolytic response in the hamster are not called upon to multiply. However, they may undergo a change in their metabolism that is not detectable morphologically. It would seem that the varied response of the glia is a function of their potential metabolic interrelationship with the neurons. That is, they do not multiply when the neurons can cope with the increased demand of trauma and survive. However, the glial cells do multiply in response to changes in those neurons that can not cope with the stress of axotomy.

In many animals, a typical glial response is an increased number of astrocytes (Torvik, 1972; Foley, 1974) following lesions resulting in neuronal death. These are the cells believed to repair defects in the CNS, i.e., they proliferate to fill the spaces that were occupied by neurons and their processes. There are many degrees of the astrocytic response, as described in the literature. Some cells form 'sheetlike' extensions around chromatolytic neurons (Torvik, 1972); others 'wall-off' an area of necrosis following a stab wound (Schultz and Pease, 1959). Most authors (Stenwig, 1972; Torvik, 1972; Watson, 1974) agree that astrocytes are responsible for the 'scar' formed following neuronal

death, whether the cells proliferate to form the scar or simply elaborate more processes.

In the hamster, the astrocytic population increased markedly in density at the highly reactive neonatal age, though the relative numbers were so small in comparison with the population of oligodendrocytes that the increase was nearly masked from a purely observational point of view. There were approximately five times more oligodendrocytes than astrocytes per sampled volume in the experimental FMN of the 1-5 dpo animals. Scanning many electron microscopic fields showed the true relative proportions of the cells, but without the differential cell counts the significant change in astrocytes would probably have been overlooked or misinterpreted.

An interesting question concerns the presence of the normal complement of oligodendrocytes in the experimental FMN of the one-day axotomy series. Cell counts would indicate that their schedule of development is unaltered and that the cells are formed and migrate to the FMN on time. These are cells which would normally myelinate the axons of the facial motor neurons. What they do when the axons they are programmed to recognize are not present is unknown. Bunge, et al., (1962) indicated that the axon must be mature in order to stimulate the oligodendrocyte to begin the process of myelination. Since there is no evidence of myelination within the glial scar as yet, it could be assumed that the oligodendrocytes, although developing in normal numbers, are simply not triggered to produce myelin. It is also possible, however, that they arise in large numbers to take part in the phagocytic response to neuronal death. They, more than

the astrocytes, seem to aggregate near the disintegrating neurons. One would have to conjecture that the metabolism of these cells had to undergo a shift, in that the function to myelinate never happens and, instead, the cells are stimulated to become phagocytic to the disintegrating neurons.

From a comparative standpoint, it is interesting to consider the types of glial responses described in human neuropathology, yet clear-cut correlations are difficult to make between the results of experimental studies like this one and the facts known about neuropathology as described in medical texts (Greenfield, 1963; Foley, 1976). Among such pathological responses are astrocytic hyperplasias and 'gitter cell' reactions (see Literature Review), each of which has been described in post-mortem or biopsy material. While the cellular identification is reproducible from case to case, there remains the question of post-mortem artifact. Human brain tissue is routinely more poorly preserved than animal tissue which has been perfusion-fixed.

The nature of the injury itself might also be a reason for differences in glial reactions. Even among experimental studies, the nature of the injury (whether an axotomy, stab wound, or nerve expulsion) determines the character of the glial response and, in fact, has been a basic source of argument concerning the origin of phagocytic cells in the affected area (see Literature Review for references). In pathological cases, injuries are neither designed nor controlled, and they may also be due to intrinsic problems that are quite different from anything inflicted experimentally. Some types of injury, such as infarcts or space-occupying lesions, may

cause widespread damage; others may be restricted to only a 'local' response of specific neurons which have been affected. Various neuropathies such as Bell's palsy are examples of 'local' injury to the brain. These responses may be linked to an immune reaction (Abramsky, et al., 1975), which further adds to the stimulus for a glial response. Little is known of the pertinent permeability and immunological conditions which exist in neuronal trauma.

Of course, the delay in collection of human material many hours after death adds artifact to the already existing neuropathological condition. It might be interesting to perform an experimental lesion on laboratory animals and collect the material at timings similar to those for autopsy specimens. Some morphological work has employed immersion fixation and has shown very dark, small glial cells (del Rio Hortega, 1932; Cammermeyer, 1963) similar to those seen in neuropathology. This type of study may also show the morphology of dying glial cells. Little is known about what these cells look like as they die or disintegrate.

Further difficulty in attempting to correlate this study with pathological findings is encountered in the response of the immature neurons. Injuries to a young human nervous system are seldom as discrete as involving only one motor nucleus. Furthermore, interactions between developing human neurons and glia can seldom be 'caught' in their early stages before total neuronal death has ensued.

One final important consideration that pathologists and experimentalists alike must approach with caution is the interpretation of cell function as based on cell appearance. Morphologists of all types

view static profiles of cells and tend to designate a cellular function based on appearance or position. For example, the cells surrounding an injured neuron are usually presumed to be preparing for phagocytosis. Might they not, instead, be supportive in a positive sense? That is, might not the glia be involved in an interchange of metabolites, or in removal of neurotoxins? Ultrastructural, biochemical and physiological studies are needed before histologically-based conclusions can be drawn.

This difficulty in correlating results of experimental alterations with the knowledge of medical pathology highlights the need for further study of both types of material. It is as important to our knowledge of the nervous system to understand the capabilities and responses of cells during stress as it is to understand their normal physiology. It is equally important to recognize that cellular capabilities change with age.

SUMMARY

The mature brain is viewed as a highly complex organ composed of two basic types of cells, the neurons and the neuroglia. Development of the brain results from the morphological and biochemical maturation of these cellular components. Morphological changes in these cells as the brain progresses from birth to the adult status have been described in this work for the facial motor nucleus (FMN) of the developing hamster. More specifically, the overall purpose was to (1) detail any glial changes which might accompany the known changes seen in normal developing neurons and (2) to describe any cytological or population changes which might reflect the glial response to neuronal trauma (axotomy) at different ages.

In the developmental portion of this work, the facial motor nuclei were studied at days 1, 5, 10, 15, and 20 after birth, as well as in the adult. Oligodendrocytes and astrocytes were identifiable throughout development on the basis of their nuclear configurations, and no age-dependent changes in their appearance were seen with light microscopy. No populations of microglia could be identified in the normal FMN at any age.

Cell counts of the FMNs showed shifts in population densities during normal development. Neurons were dispersed with growth and, therefore, decreased markedly in number per sampled volume. Oligodendrocytes were the most numerous cell type at all ages. They underwent a dramatic increase in population density between birth and

5 days, at a time immediately preceding the onset of myelination, and then gradually decreased in density as development proceeded. The astrocytic population was very small by comparison but showed a progressive rise in density throughout development.

When the facial nerve was severed in adult hamsters and at 1, 5, 10, 15, and 20 days of age, marked differences in glial response could be directly correlated with age-dependent differences in neuronal response. Axotomy at 1 day of age resulted in rapid neuronal death, accompanied by a marked increase in the population density of both astrocytes and oligodendrocytes. The same operation at 5 or 10 days resulted in much less neuronal death and only transient changes in the glial populations. Axotomy at older ages, including the adult, produced only transient chromatolysis in the neurons and no changes in the glial counts. No histological changes in glial cell appearance were observed at any age with light microscopy, in either short-term or long-term postoperative facial nuclei. Also, no population of microglial cells was found.

The ultrastructural portion of this work verified the identification of the glial elements in the normal FMN. Again, no populations of normal microglia could be found. Furthermore, in the experimental adult material no overt changes could be seen in the glial morphology as a result of axotomy. A study of the highly reactive period 3 days after axotomy at 1 day of age, however, revealed details not discernible with light microscopy. Not only could disintegration of neurons into electron dense debris be viewed ultrastructurally, but some evidence of phagocytic activity on the part of glial cells was also found. The

reactive type of cell bore some resemblance to both oligodendrocytes and microglial cells. Both normal astrocytes and oligodendrocytes were also seen, as were 'blast' stages of both cell types.

Comparisons have been made between these results in the developing hamster and those in other species. An increase in the density of glial populations following axotomy in older animals may hold true for rats and mice, but not hamsters, because of the greater amount of neuronal death in these other species. The greater resistance of adult hamster neurons to injury and stress may be due to their more well developed capacity to synthesize the proteins needed for repair, as evidenced by their more advanced nucleolar structure. It is probable that the neurons of young animals, on the other hand, are more vulnerable to axotomy because of their lack of fully matured capacity to produce sufficient necessary proteins.

The role of glial cells in relation to neurons is still a matter for conjecture. It seems possible that they may be supportive in the sense of metabolic interchange and that their increase in numbers accompanying neuronal death may represent an attempt to compensate for neuronal inability to cope with the stress. Even in situations where no histological response is evident, there may be cellular interchanges on a biochemical level.

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FIGURE 1

Comparison of faces of normal and operated adult hamsters.

A. Normal animal.

B. Experimental animal, 100 days after axotomy of the right nerve. The operation was performed at 1 day of age.

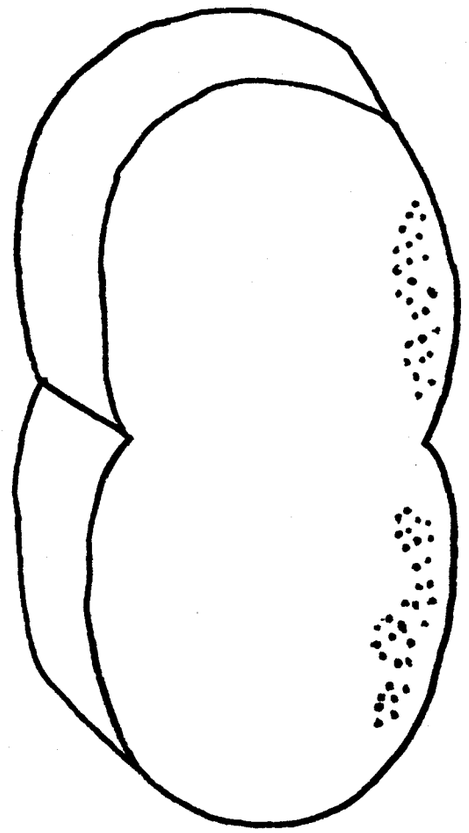
A

B

FIGURE 2

Diagram of the location of the facial motor nucleus at the ponto-medullary level of the brain stem. Dark circles denote the position of the cell bodies.

V = Ventral D = Dorsal



LOCATION OF THE FACIAL MOTOR NUCLEUS IN THE BRAIN STEM

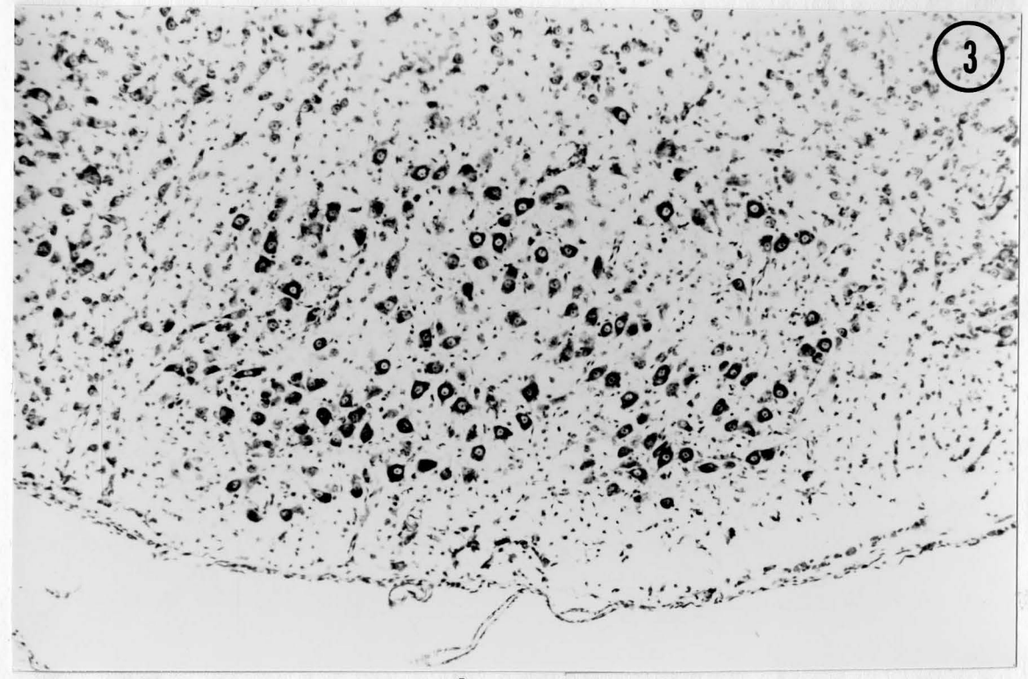
(2)

FIGURE 3

Facial motor nuclei of normal hamster brain. Thionin stain.

A. Adult (110X).

B. Newborn (575X).



A

B

FIGURE 4

Representative cell types found within the facial motor nucleus of the normal adult hamster. Thionin stain. (1440X)

Key to abbreviations used throughout figures.

N = Neuron

O = Oligodendrocyte

A = Astrocyte

BV = Blood vessel

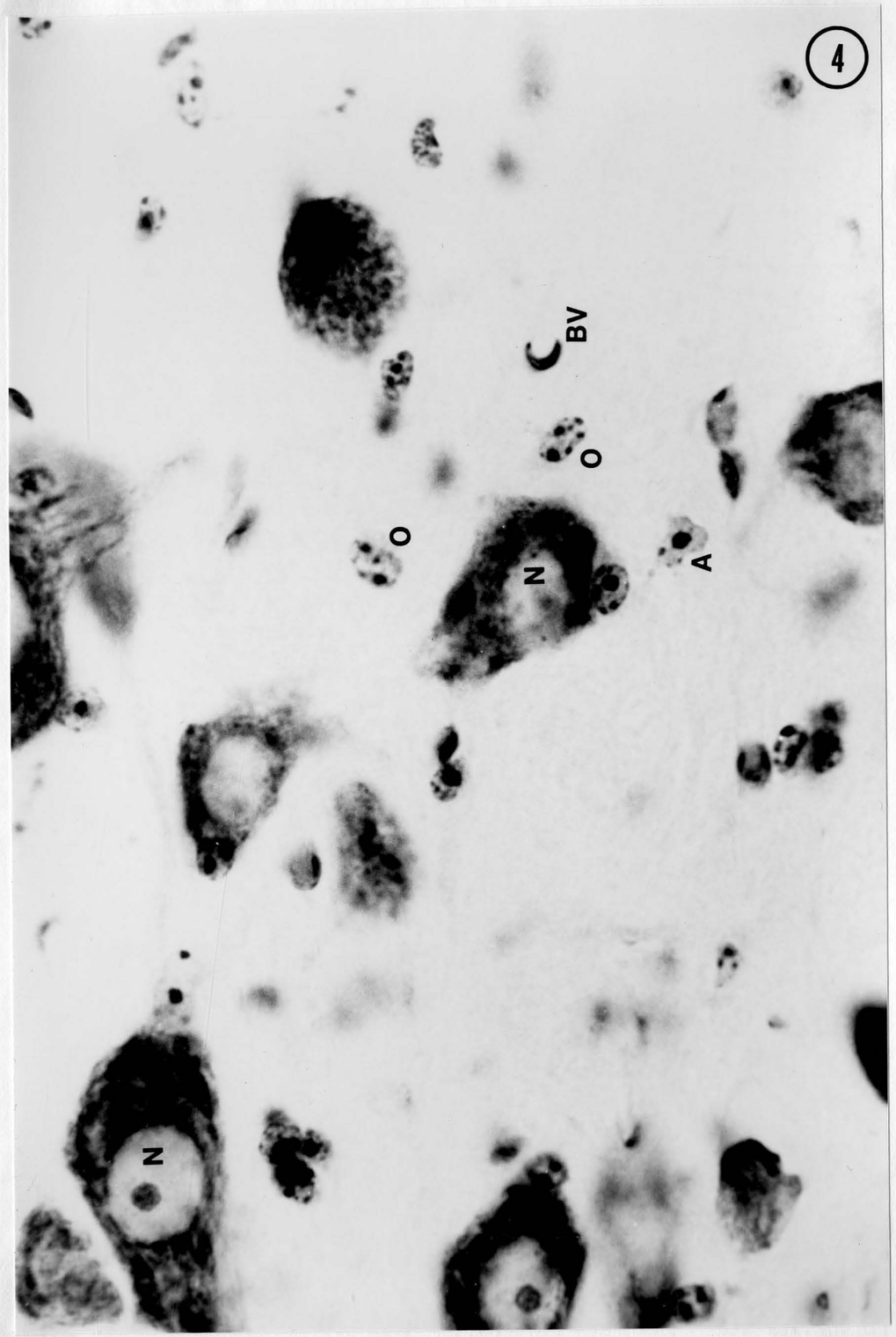
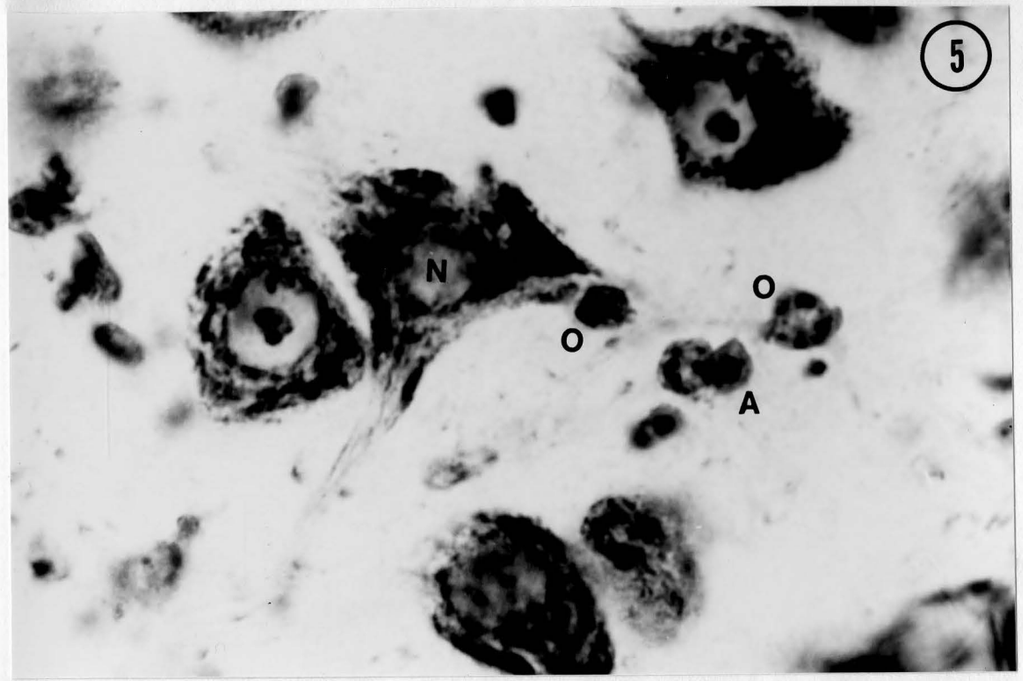


FIGURE 5

Representative cell types found within the facial motor nucleus of the normal developing hamster. Thionin stain. (1440X)

A. 20 day old hamster. Note similarity to adult (Fig. 4).

B. 15 day old hamster. Note the dark staining of the neurons, indicating a temporary greater density of Nissl substance at this age.



A

B

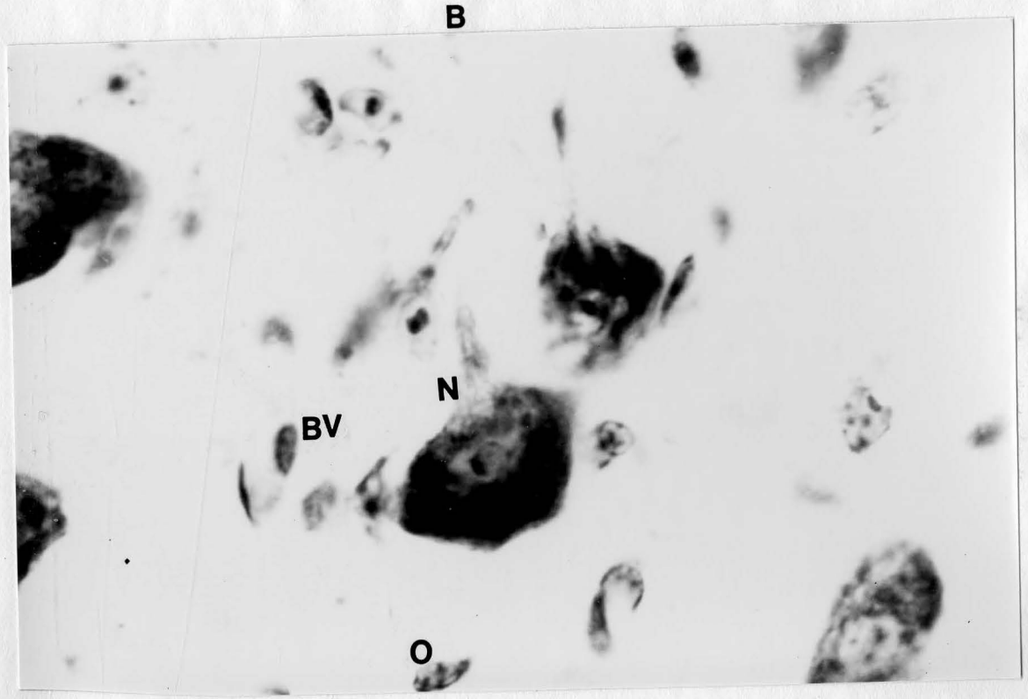
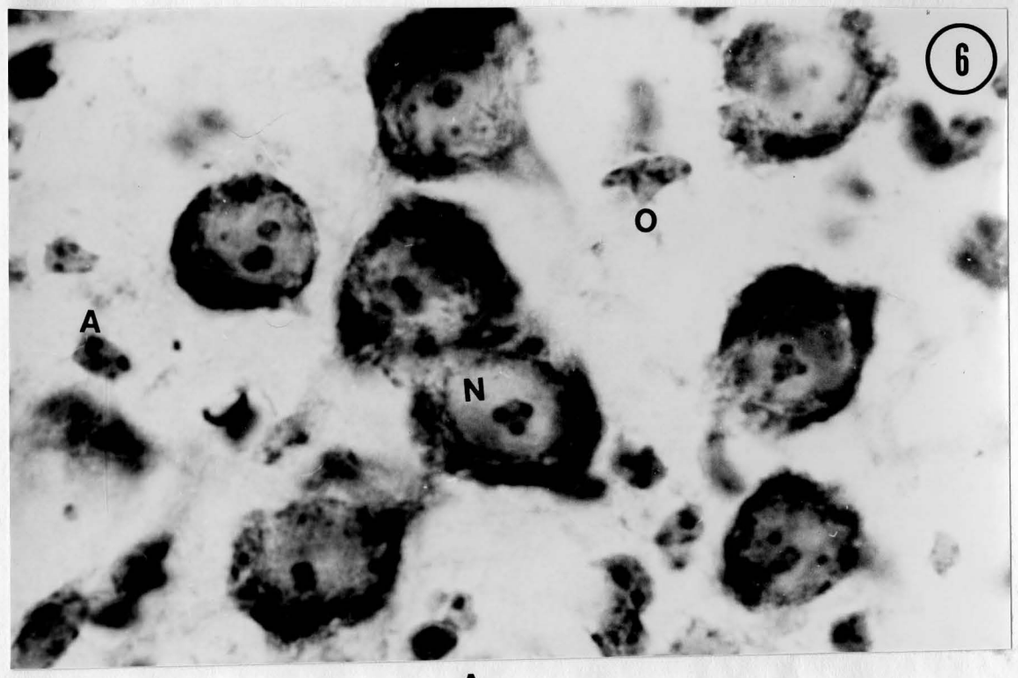


FIGURE 6

Representative cell types found within the facial motor nucleus of the normal developing hamster. Thionin stain. (1440X)

A. 5 day old hamster. Note similarity to adult configuration already. (See Fig. 4).

B. Newborn hamster. Note multiple nucleoli and general immaturity as compared with 5 days.



A

B

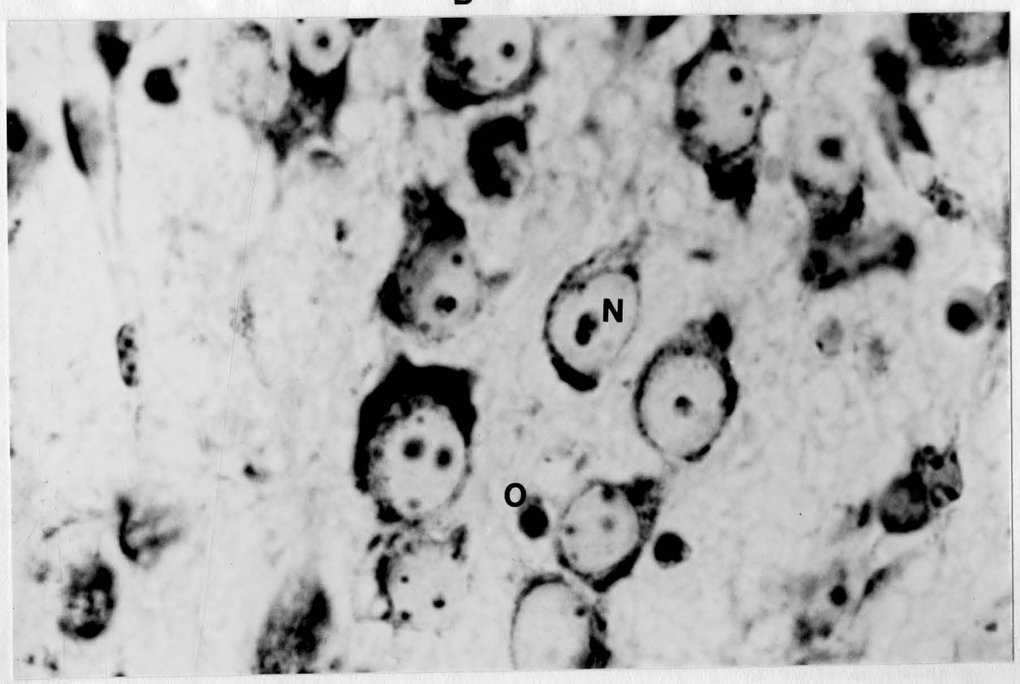
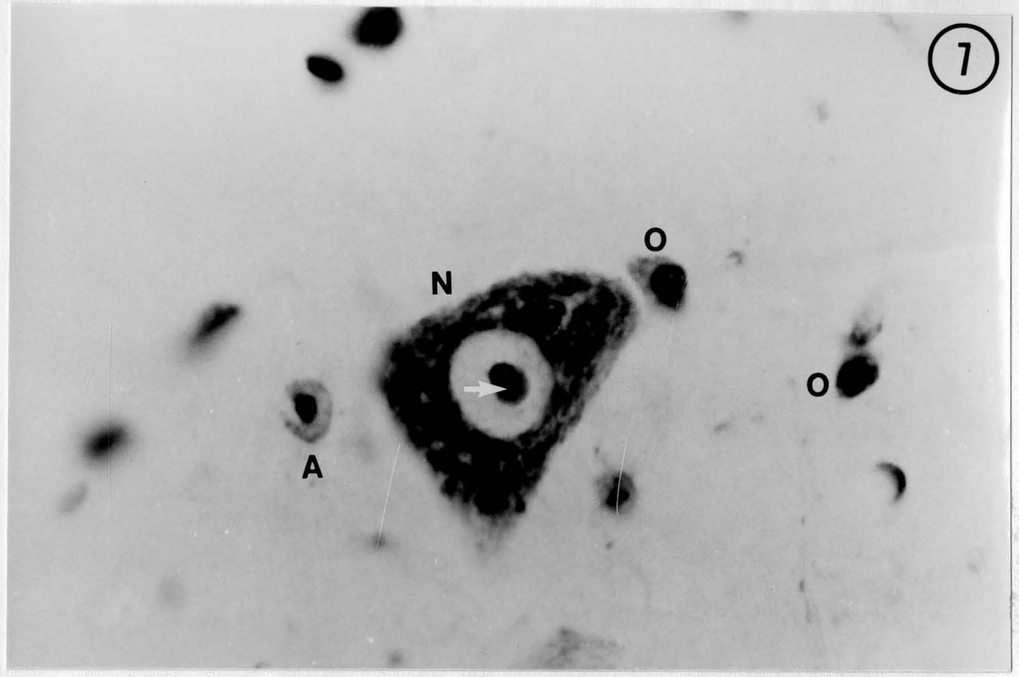


FIGURE 7

Comparison of normal and experimental facial motor nuclei 5 days after axotomy in the adult hamster. Thionin stain. (1440X)

A. Normal side. Note prominent intranucleolar body indicated by arrow.

B. Experimental side. Note somal swelling, diffuse chromatolysis and absence of intranucleolar body.



A

B

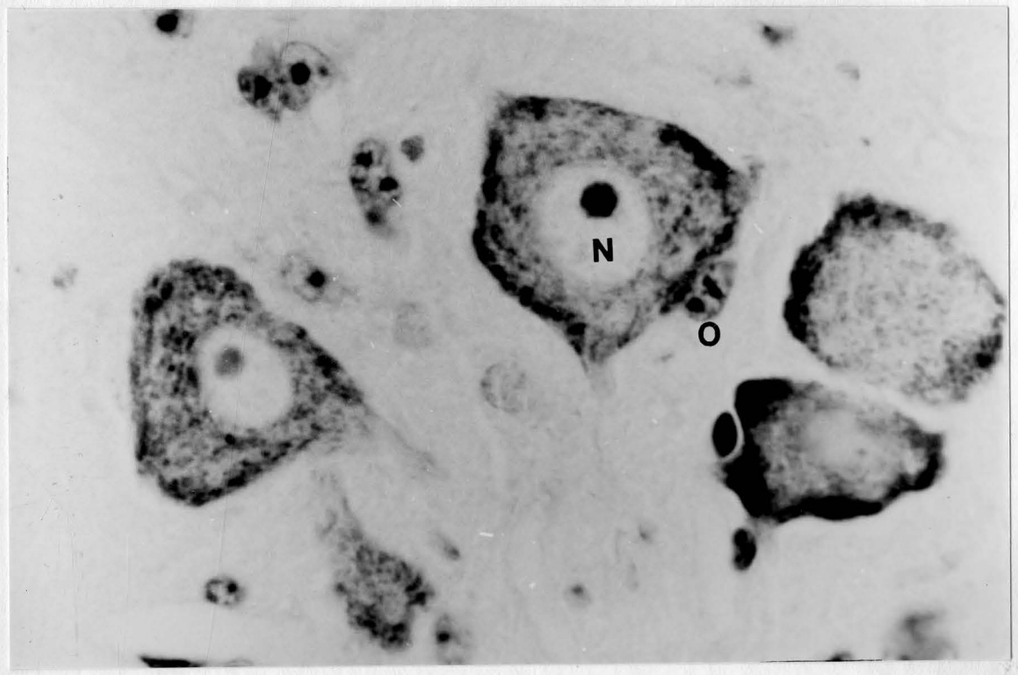


FIGURE 8

Experimental facial motor nucleus 20 days after axotomy in the adult hamster. Compare with Figure 2 for normal adult appearance. Thionin stain. (1440X)

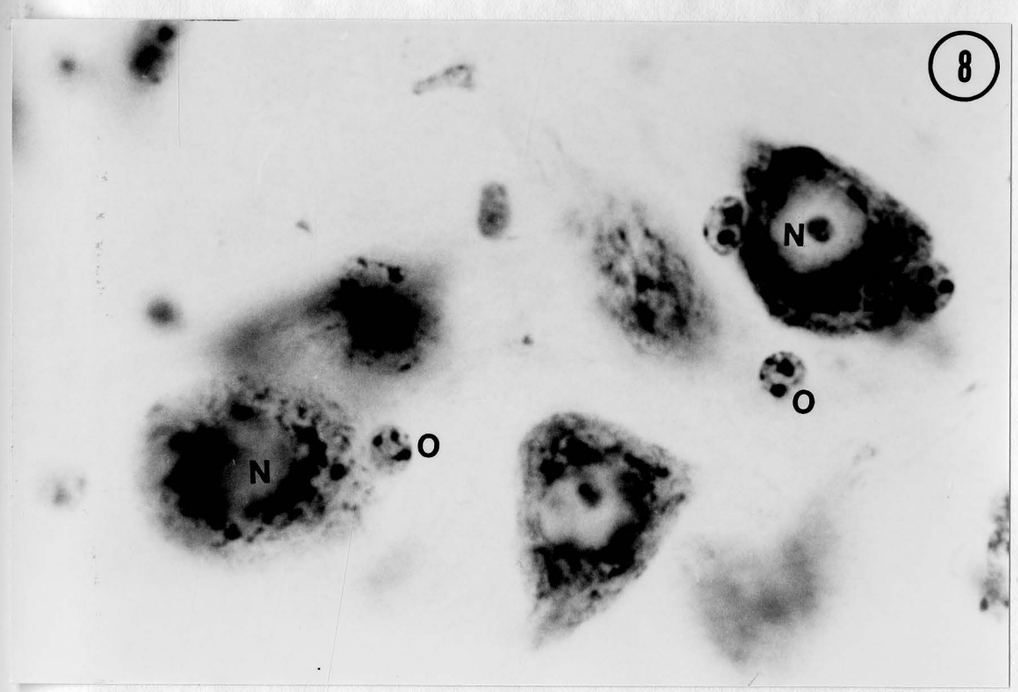
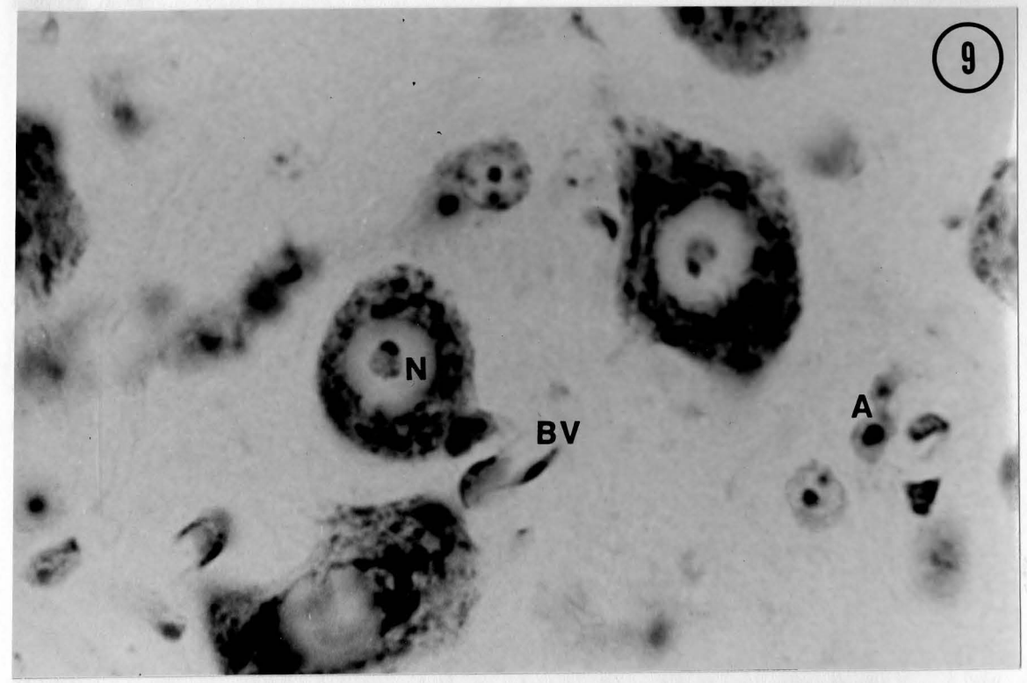


FIGURE 9

Comparison of normal and experimental facial motor nuclei 10 days after axotomy in the 20 day old hamster.

A. Normal side.

B. Experimental side. Note diffuse chromatolysis accompanied by somal swelling.



A

B

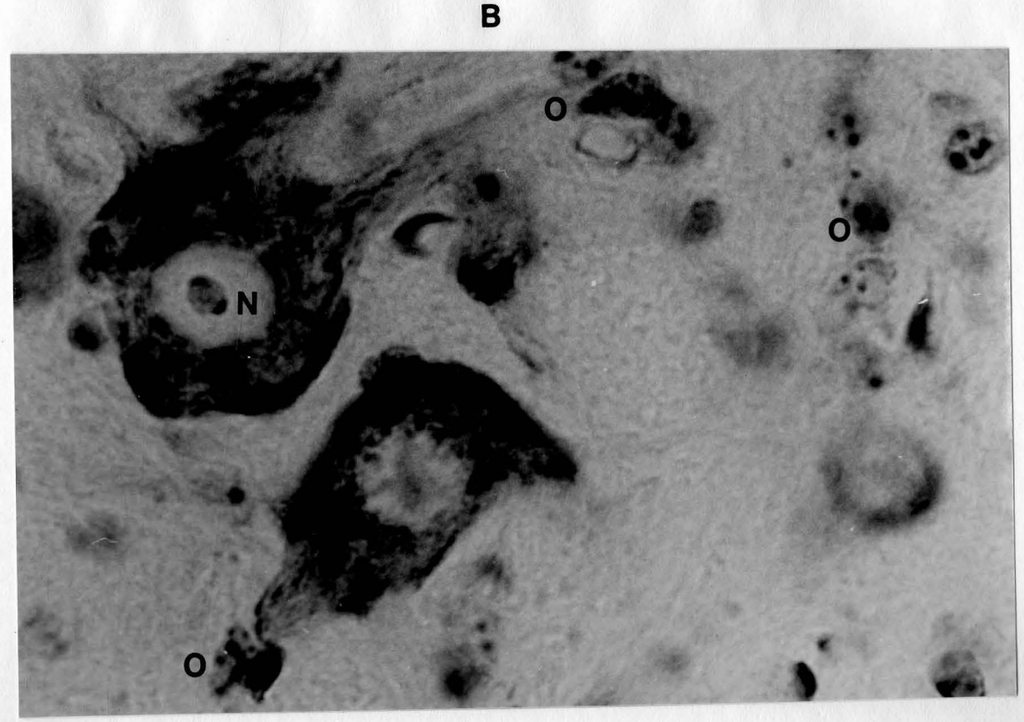
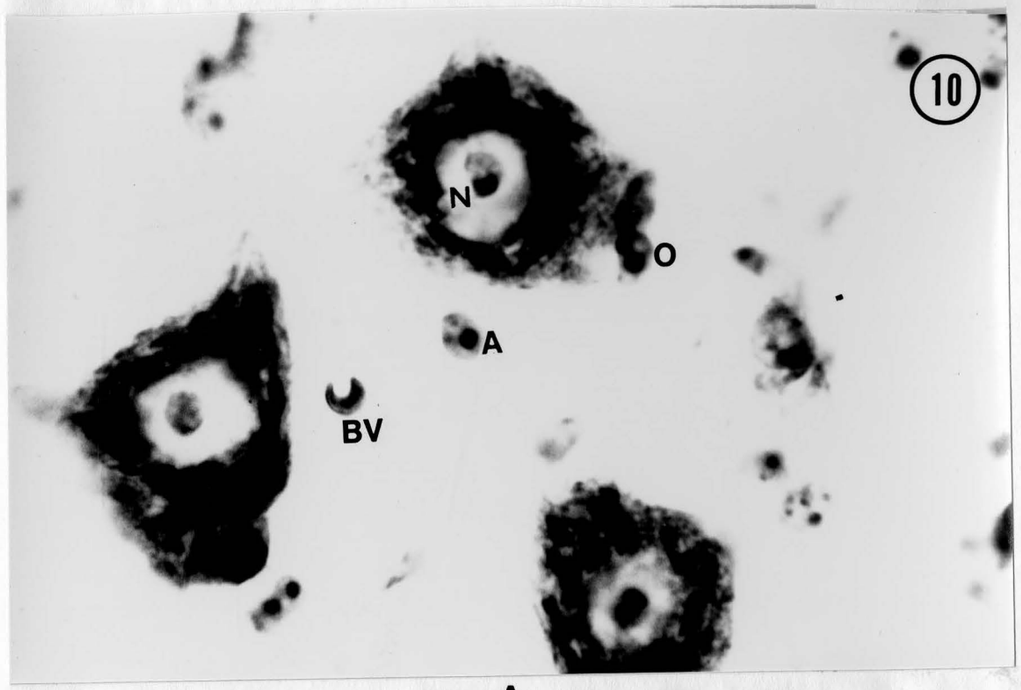


FIGURE 10

Comparison of normal and experimental facial motor nuclei 5 days after axotomy in the 15 day old hamster. Thionin stain. (1440X)

A. Normal side.

B. Experimental side. Note 'focal' chromatolysis (arrow) without cellular swelling.



A

B

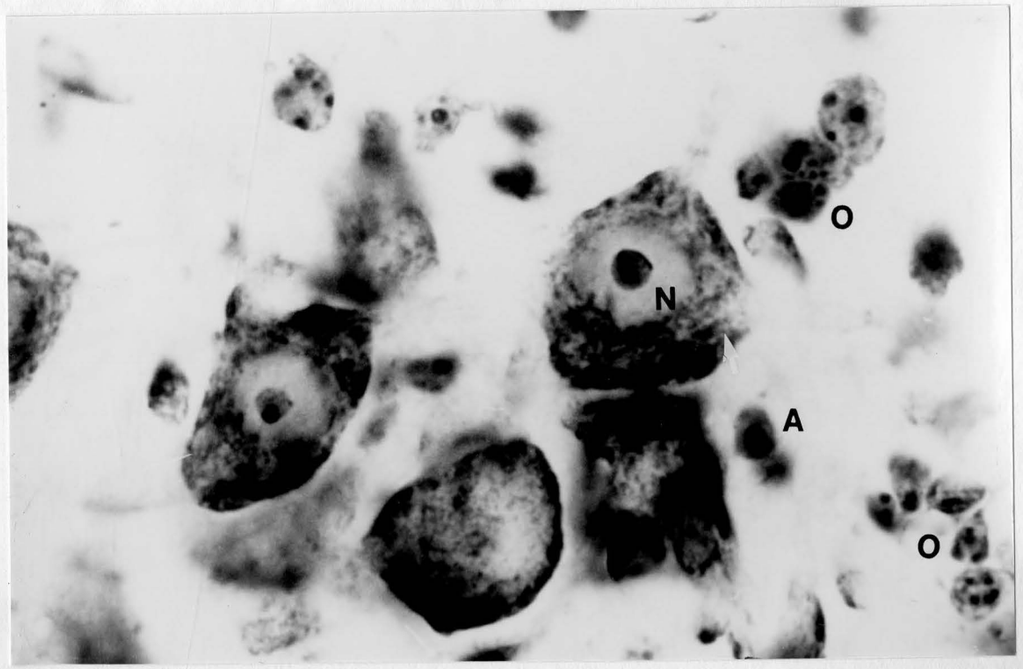
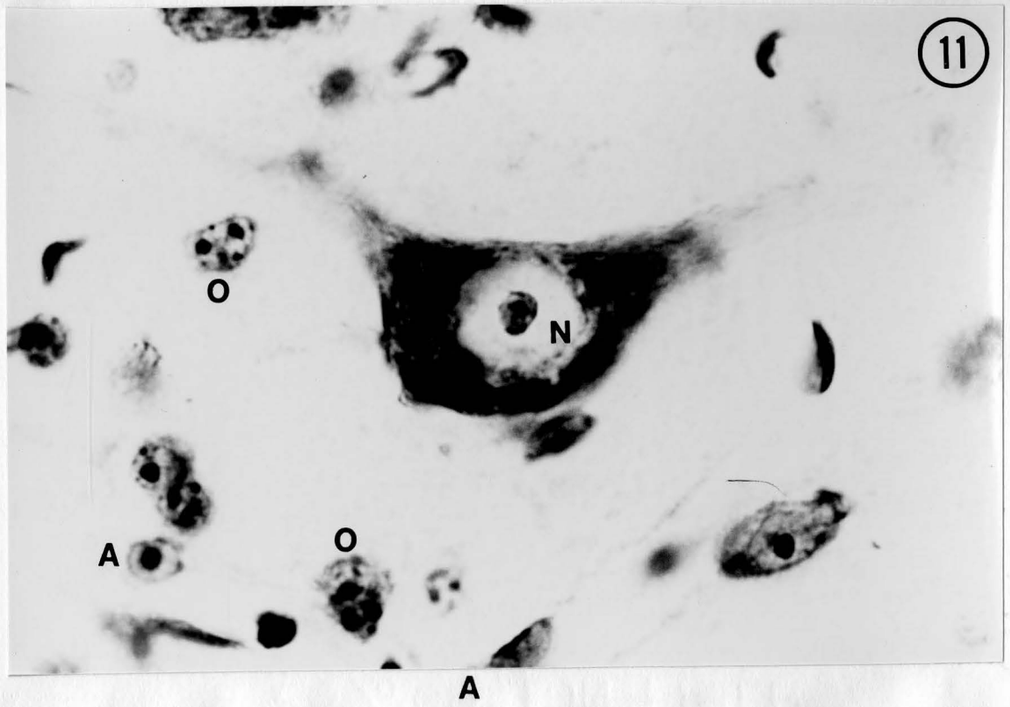


FIGURE 11

Comparison of normal and experimental facial motor nuclei 20 days after axotomy in the 10 day old hamster. Thionin stain. (1440X)

A. Normal side.

B. Experimental side. Note that some 'focal' chromatolysis still remains (arrow).



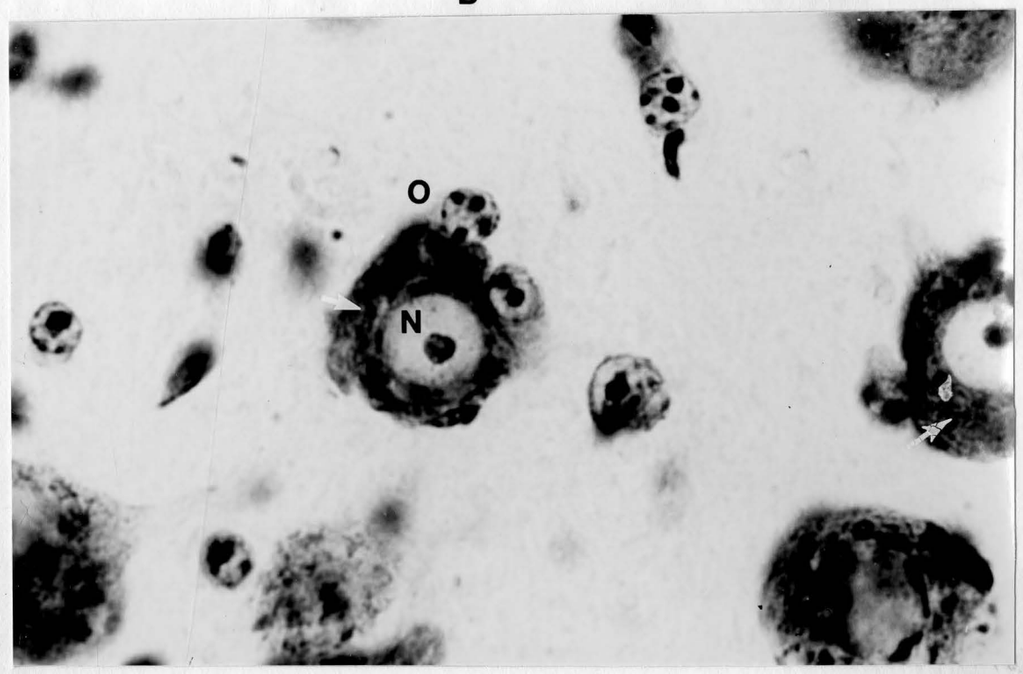


FIGURE 12

Comparison of normal and experimental facial motor nuclei 5 days after axotomy in the 5 day old hamster. Thionin stain. (1440X)

A. Normal side.

B. Experimental side. Note chromatolysis and regression of somal size.

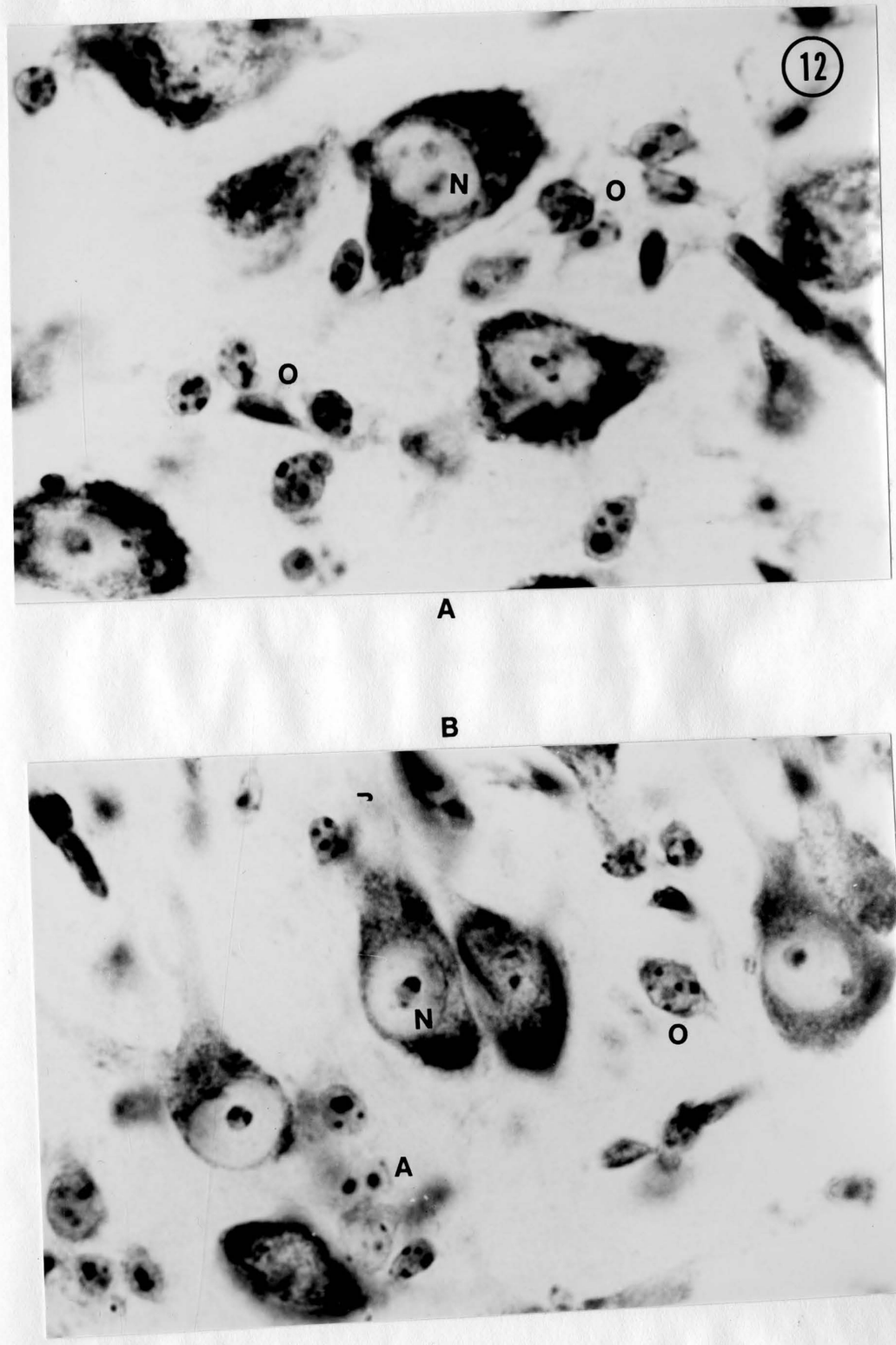
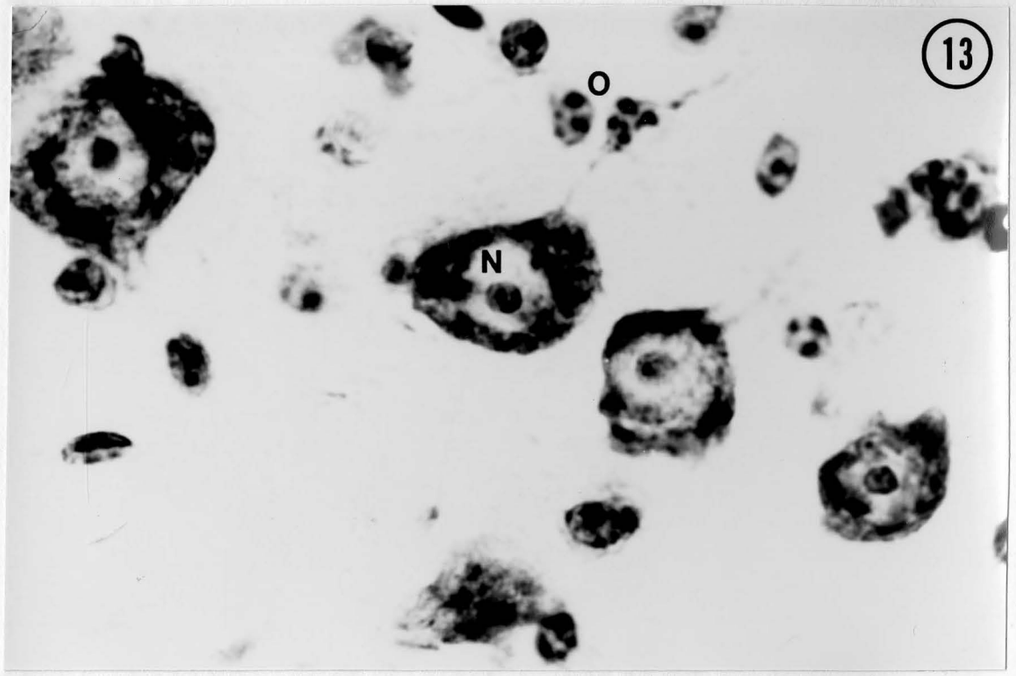


FIGURE 13

Comparison of normal and experimental facial motor nuclei 100 days after axotomy in the 5 day old hamster. Thionin stain. (1440X)

A. Normal side.

B. Experimental side. Note the absence of large neurons.



A

B

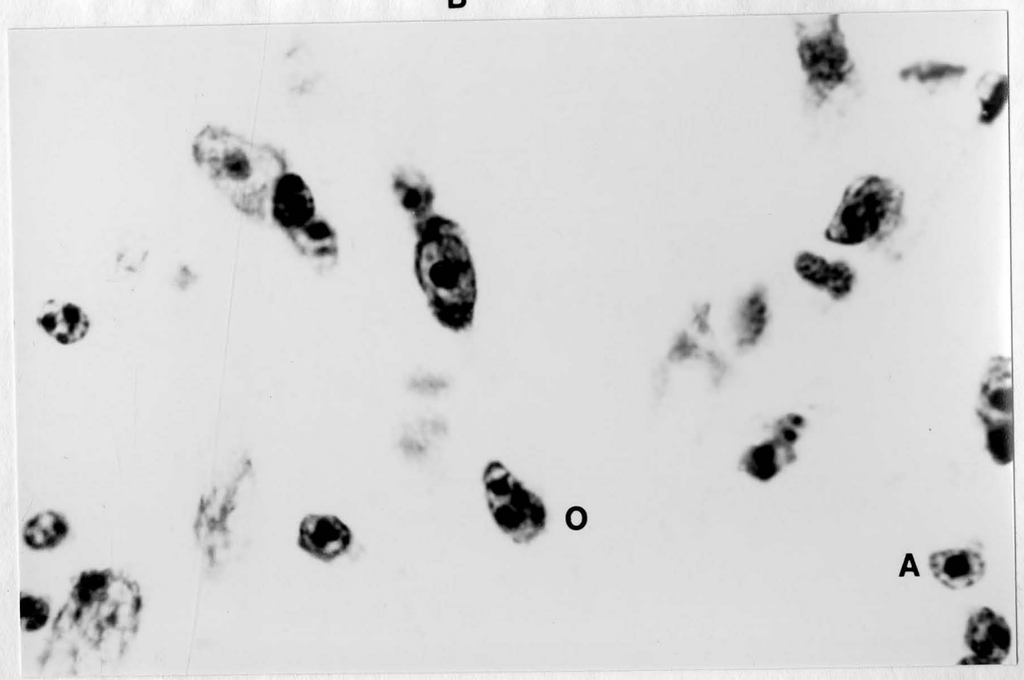


FIGURE 14

Comparison of normal and experimental facial motor nuclei 50 days after axotomy in the 1 day old hamster. Thionin stain. (1440X)

A. Normal side.

B. Experimental side. Note the absence of large neurons. Only glia remain.

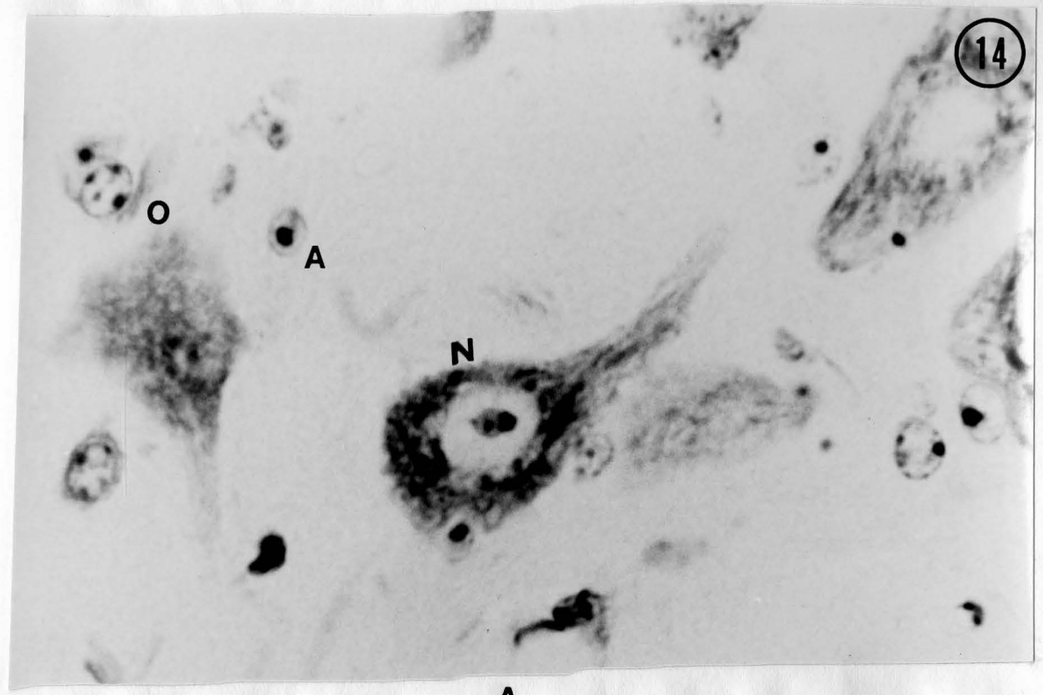


FIGURE 15

Brain stem containing normal and experimental facial motor nuclei 10 days after axotomy in the 1 day old hamster. Thionin stain. (320X)

A. Normal side.

B. Experimental side. Note the overall lack of neurons even at this low power.

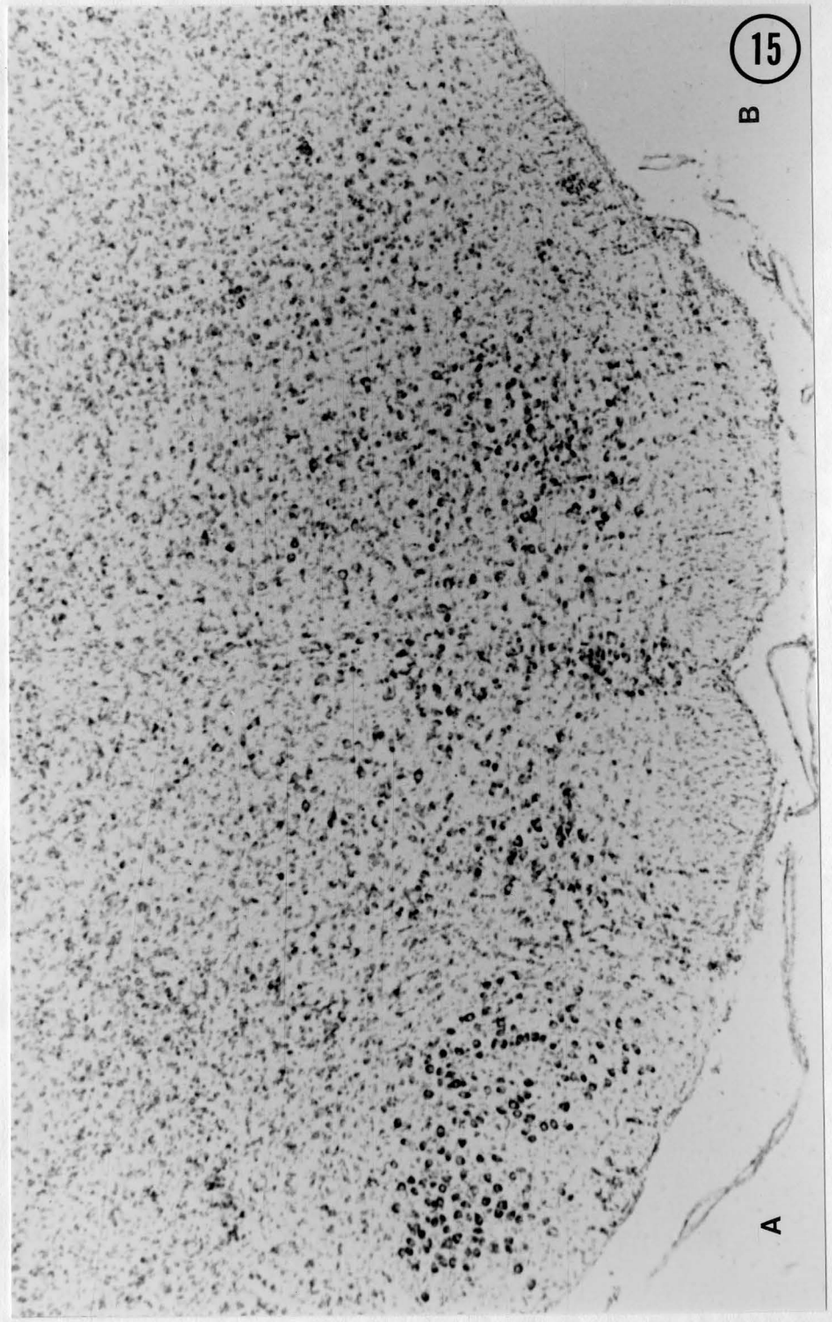


FIGURE 16

Comparison of normal and experimental facial motor nuclei 30 days after axotomy in the adult mouse. Thionin stain. (110X)

A. Normal side.

B. Experimental side. Note decreased number of neurons.

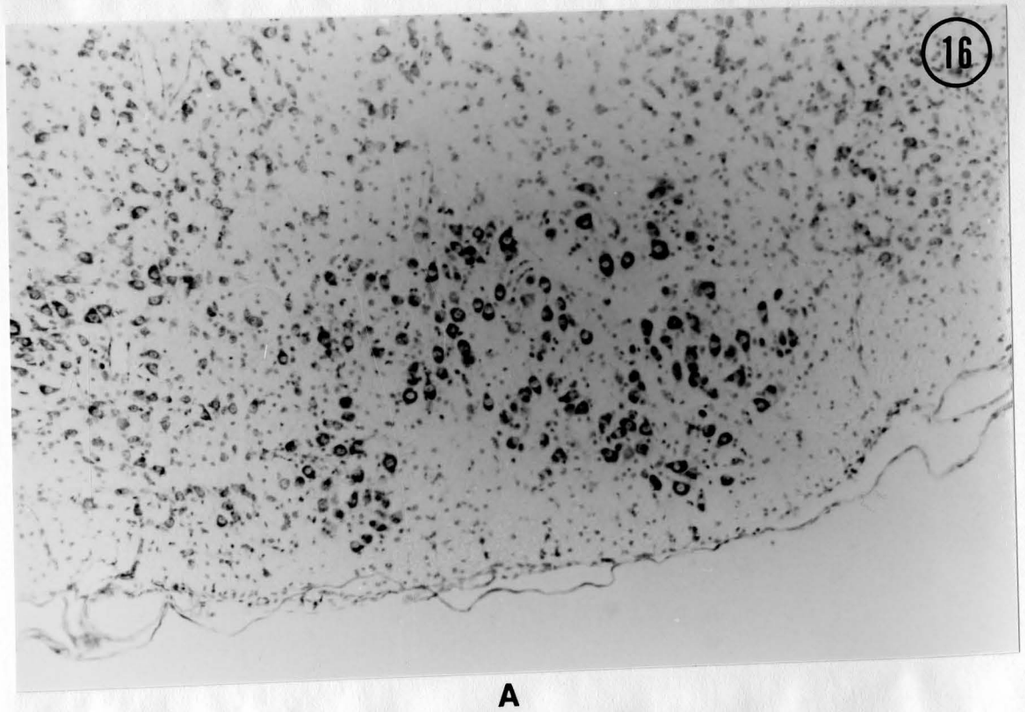
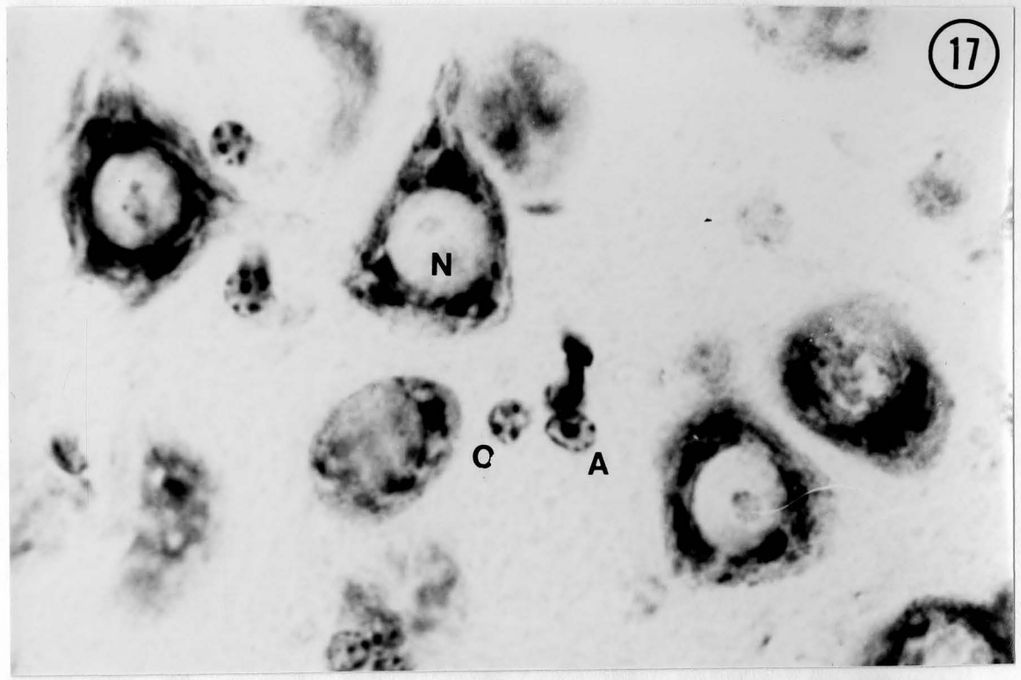


FIGURE 17

Comparison of normal and experimental facial motor nuclei 30 days after axotomy in the adult mouse. Thionin stain. (1440X)

A. Normal side.

B. Experimental side. Note the chromatolytic appearance of these neurons.



A

B

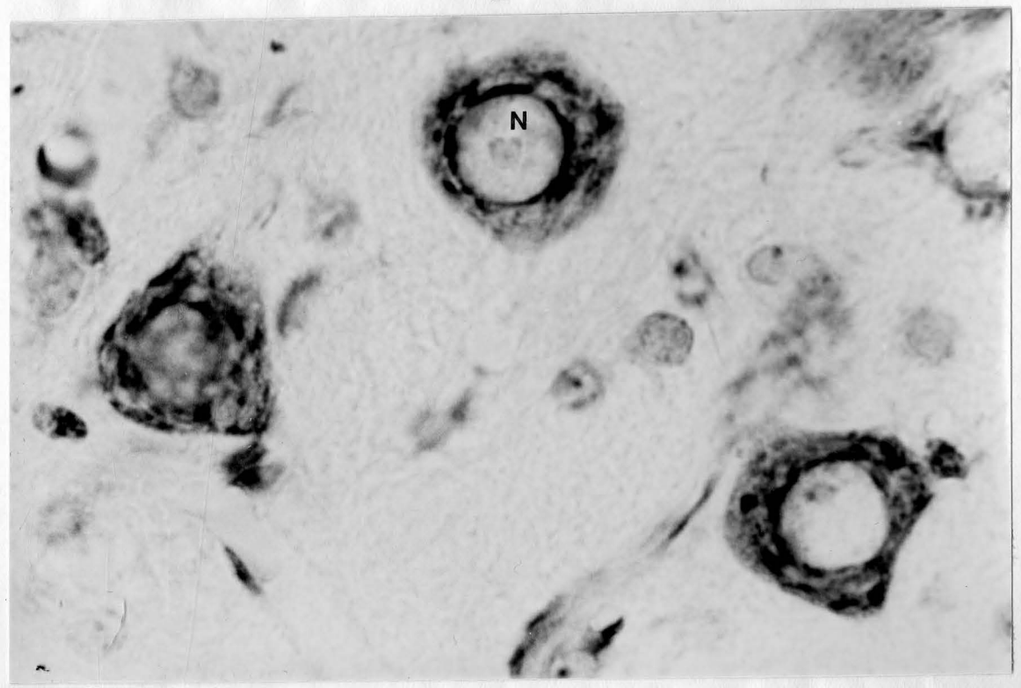


FIGURE 18

Graph representing the cell counts performed in the facial motor nucleus of the developing hamster. Each bar represents the mean ± 2 standard errors.

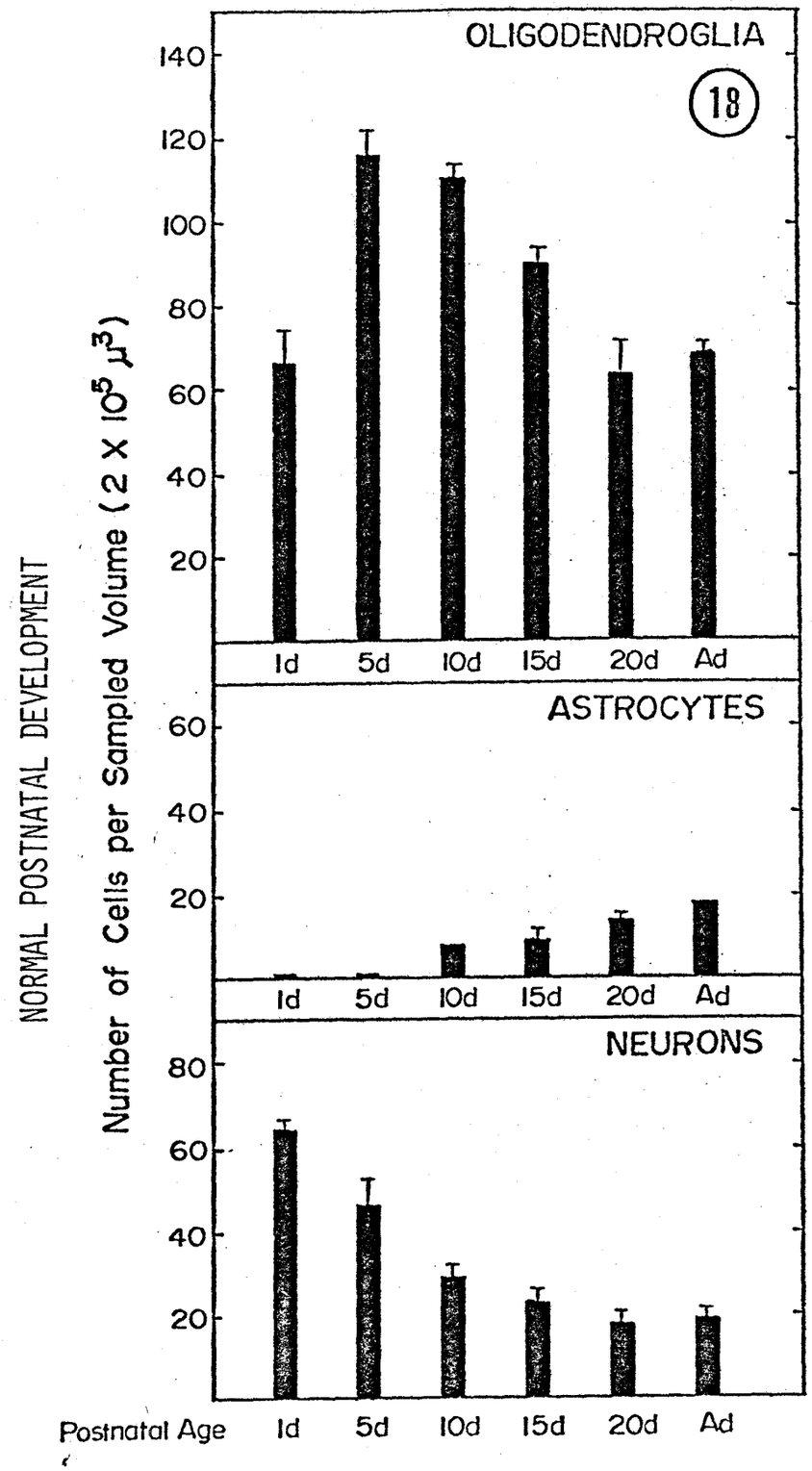


FIGURE 19

Graph representing cell counts performed at different ages in the facial motor nucleus of the hamster following axotomy at 1 day of age. Each bar represents the mean ± 2 standard errors.

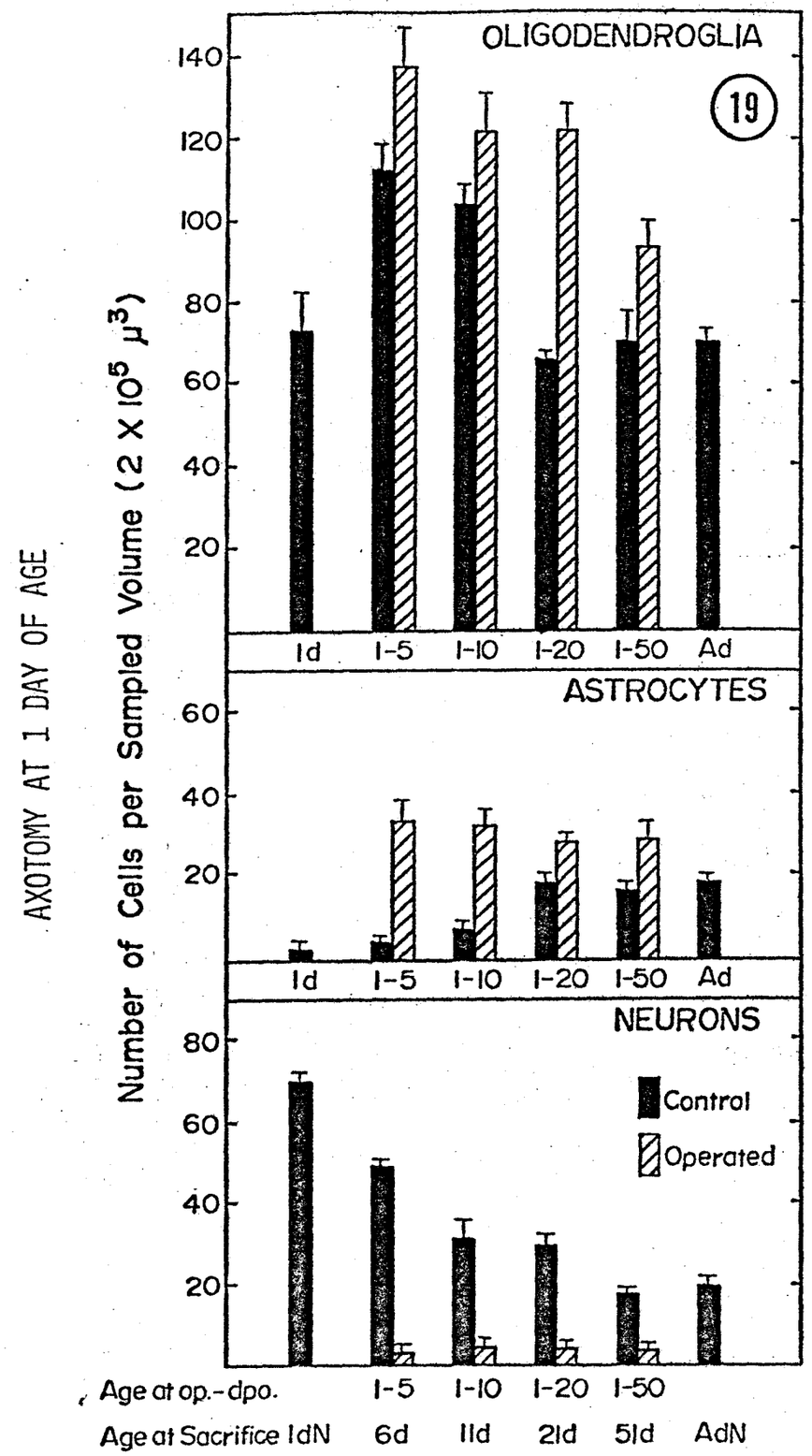


FIGURE 20

Graph representing cell counts performed at different ages in the facial motor nucleus of the hamster following axotomy at 5 days of age. Each bar represents the mean ± 2 standard errors.

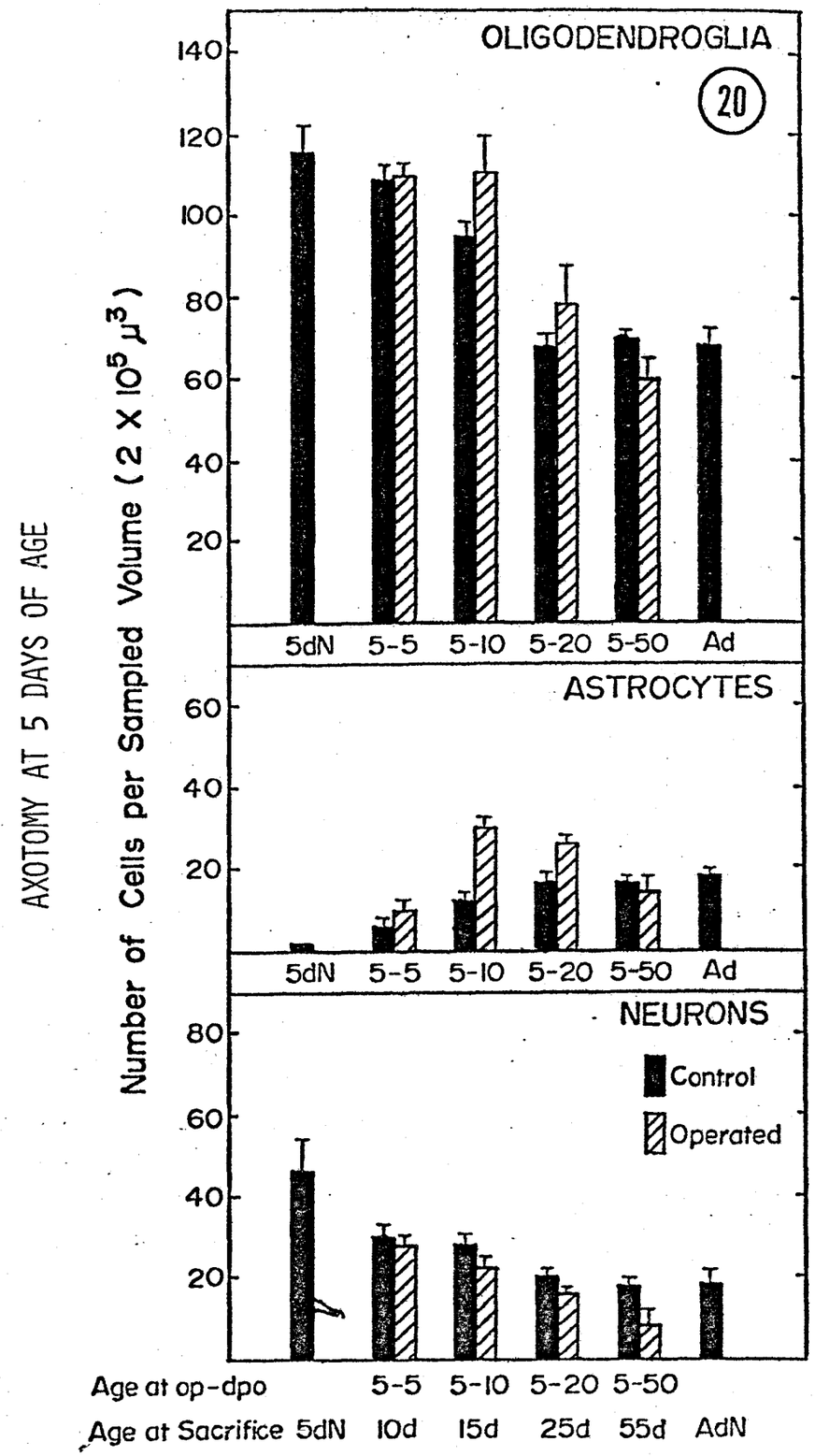


FIGURE 21

Graph representing cell counts performed at different ages in the facial motor nucleus of the hamster following axotomy at 10 days of age. Each bar represents the mean ± 2 standard errors.

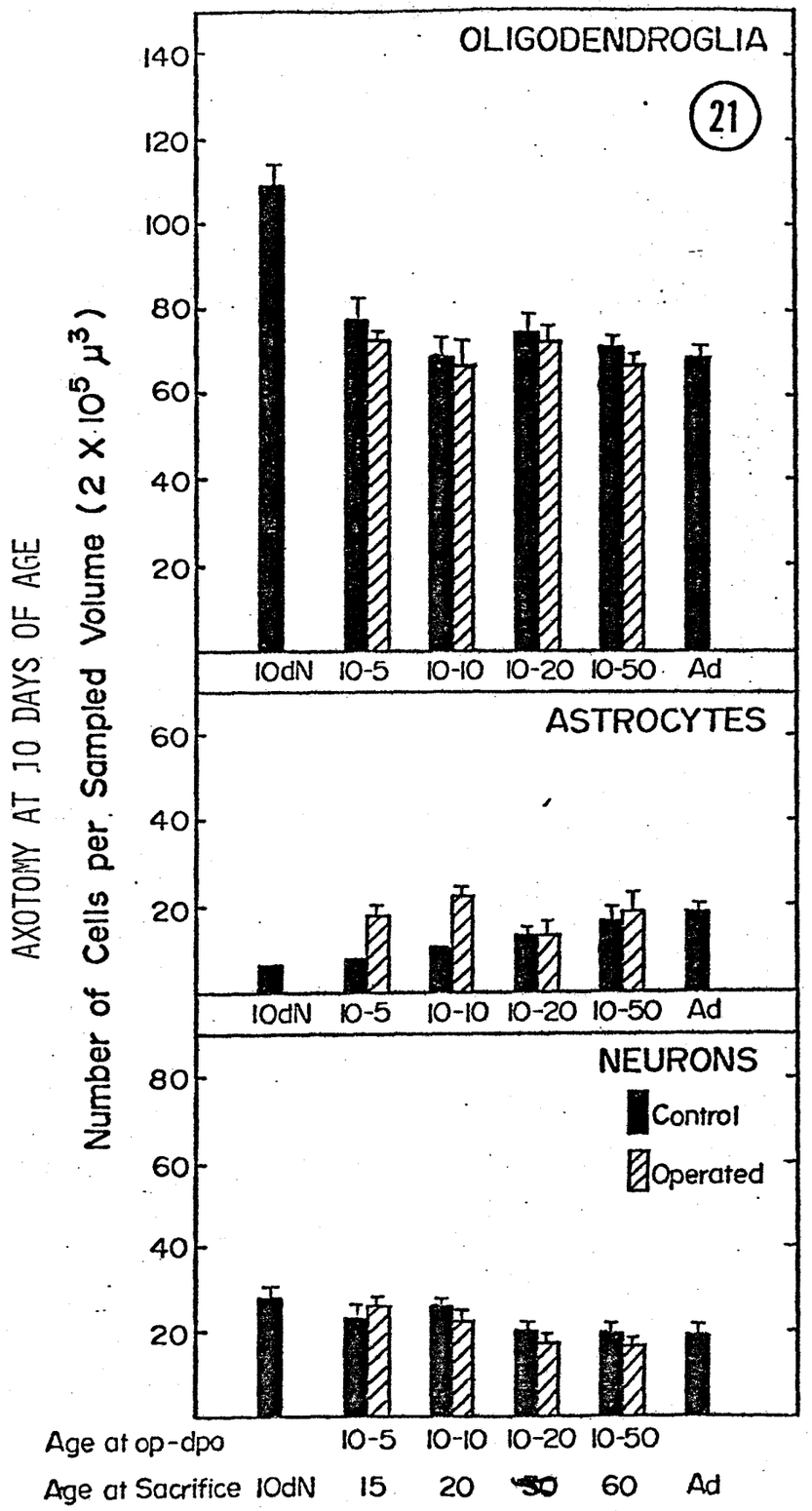


FIGURE 22

Graph representing cell counts performed at different ages in the facial motor nucleus of the hamster following axotomy at 15 days of age. Each bar represents the mean ± 2 standard errors.

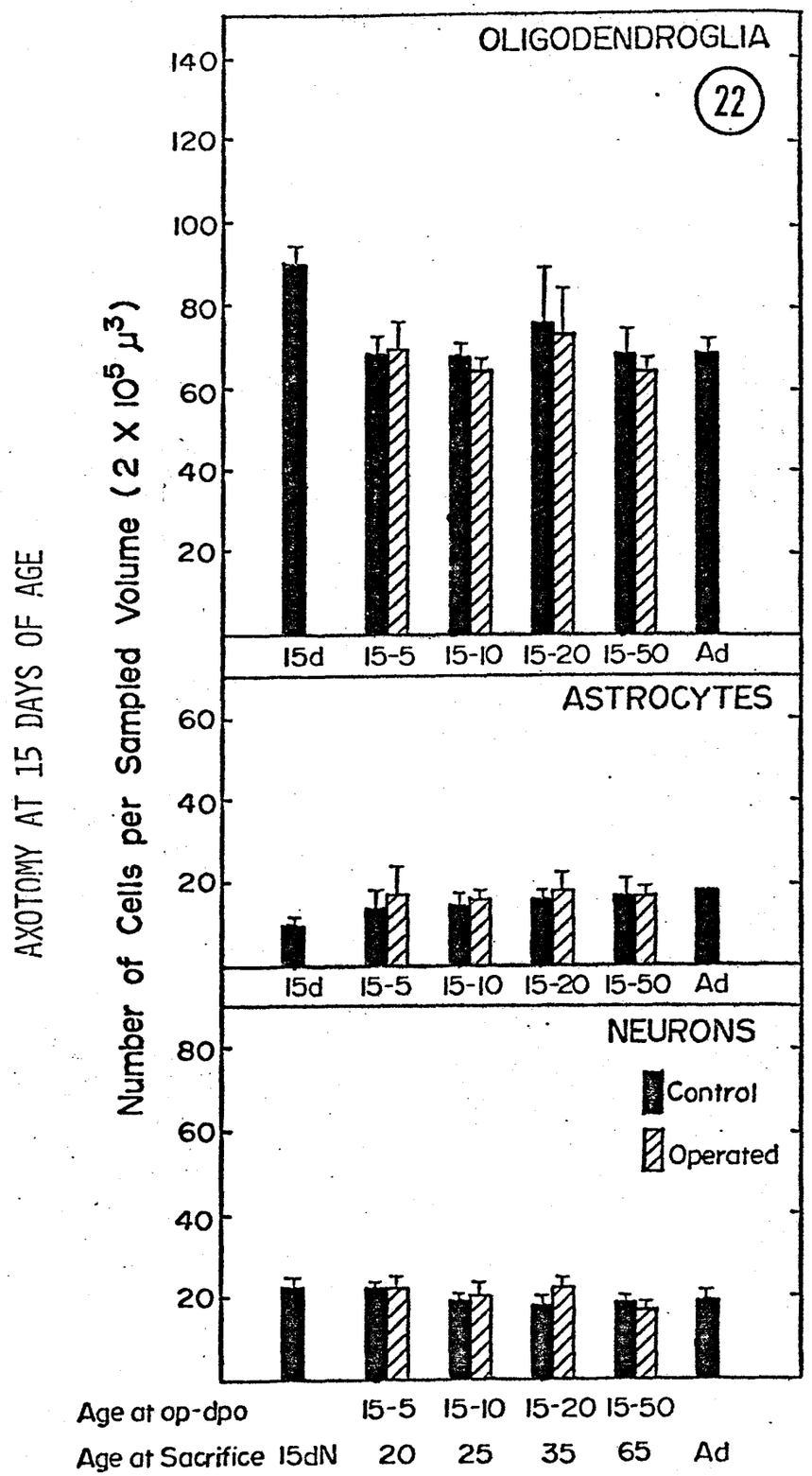


FIGURE 23

Graph representing cell counts performed at different ages in the facial motor nucleus of the hamster following axotomy at 20 days of age. Each bar represents the mean ± 2 standard errors.

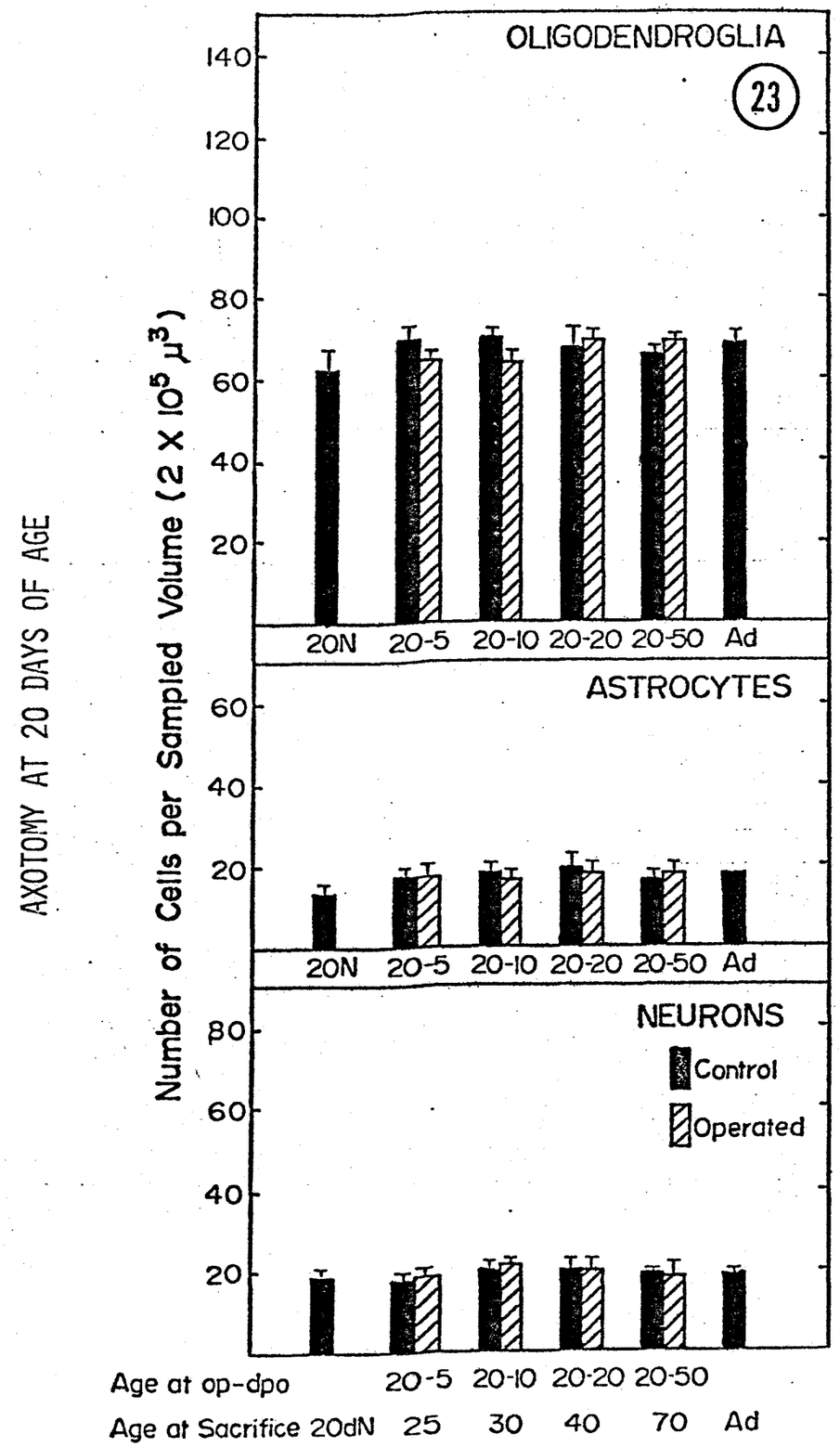


FIGURE 24

Graph representing cell counts performed at different ages in the facial motor nucleus of the hamster following axotomy in the adult. Each bar represents the mean ± 2 standard errors.

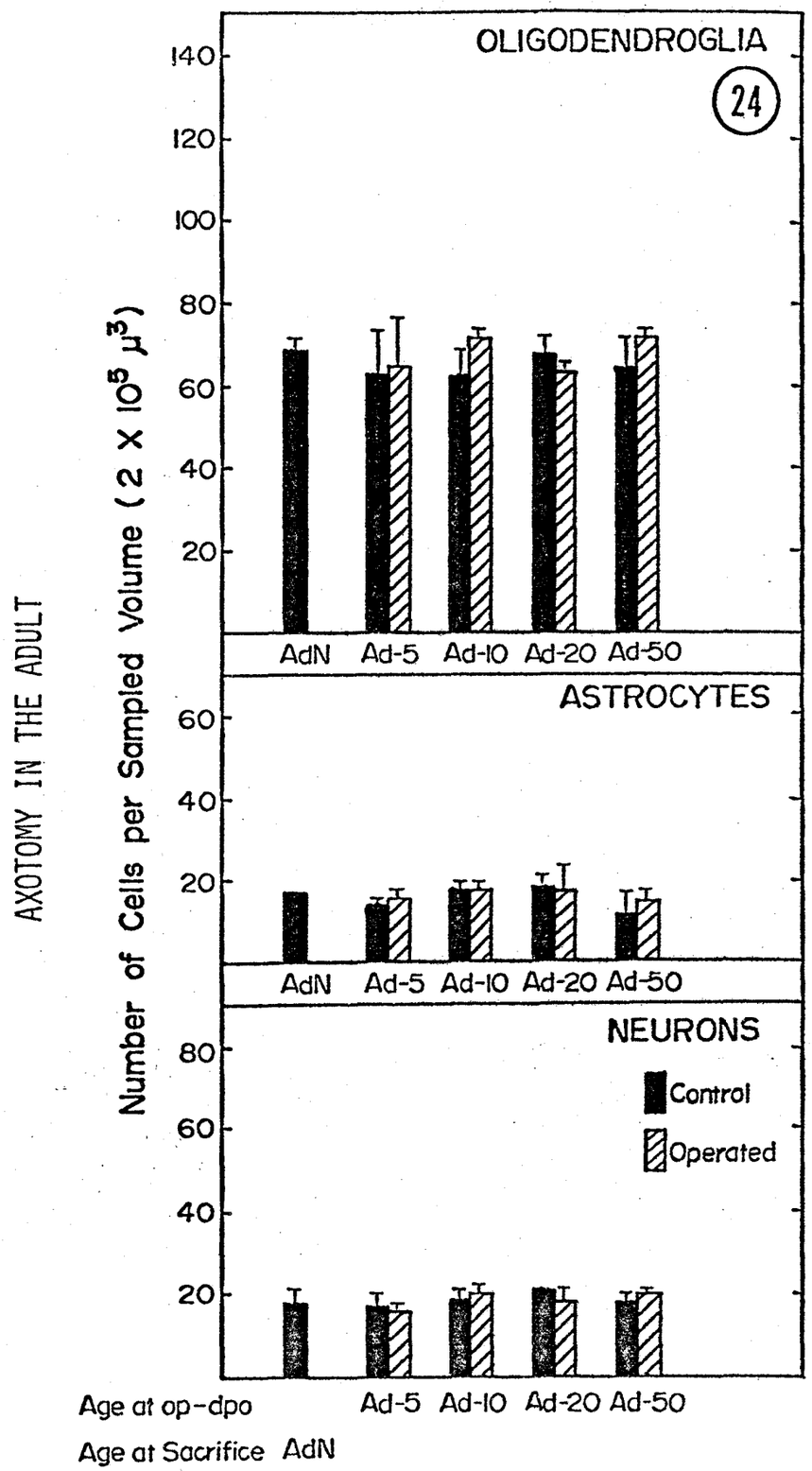


FIGURE 25

Section of facial motor nucleus of the adult hamster. MP = myelinated processes. Plastic thick section. Toluidine blue. (575X)

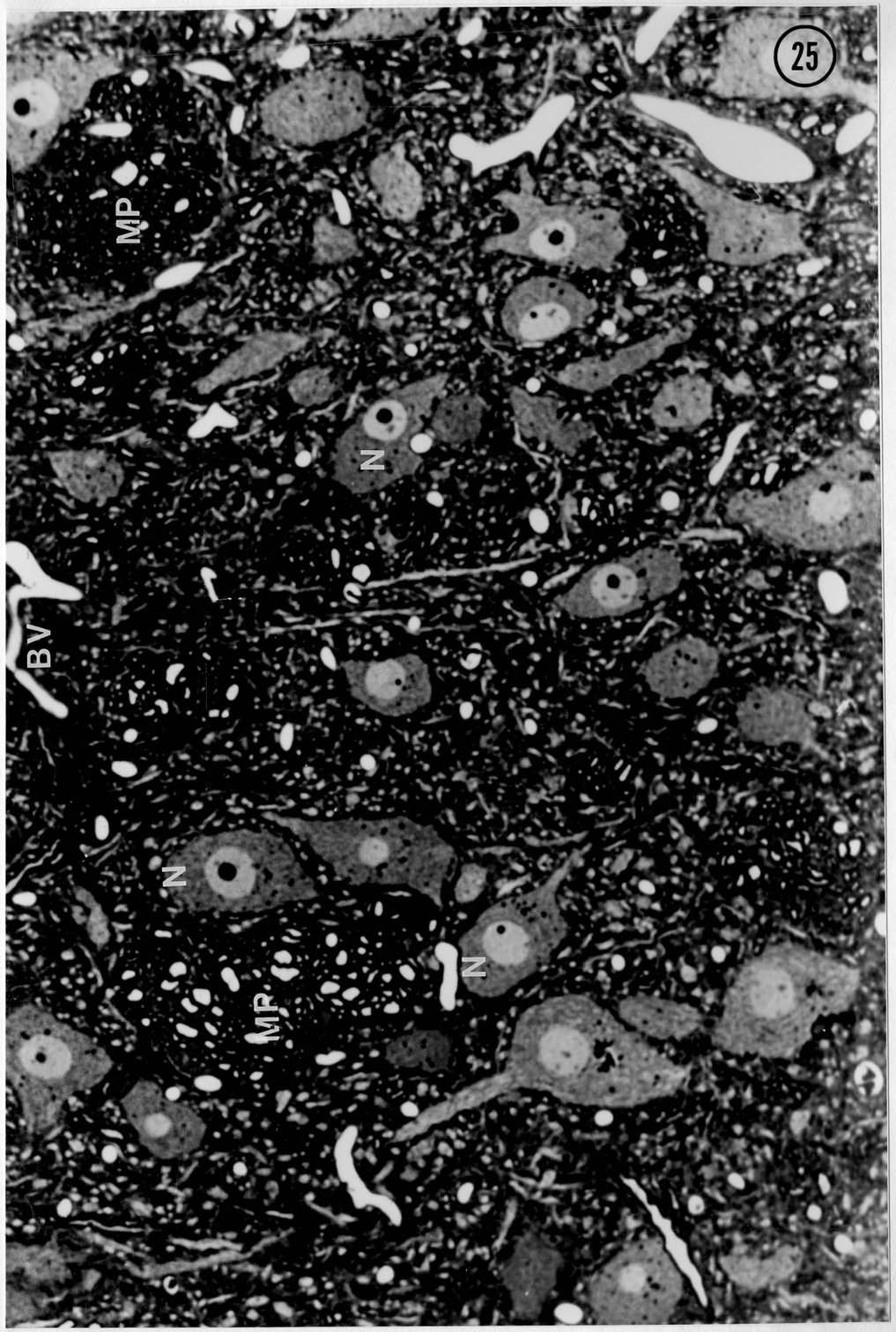
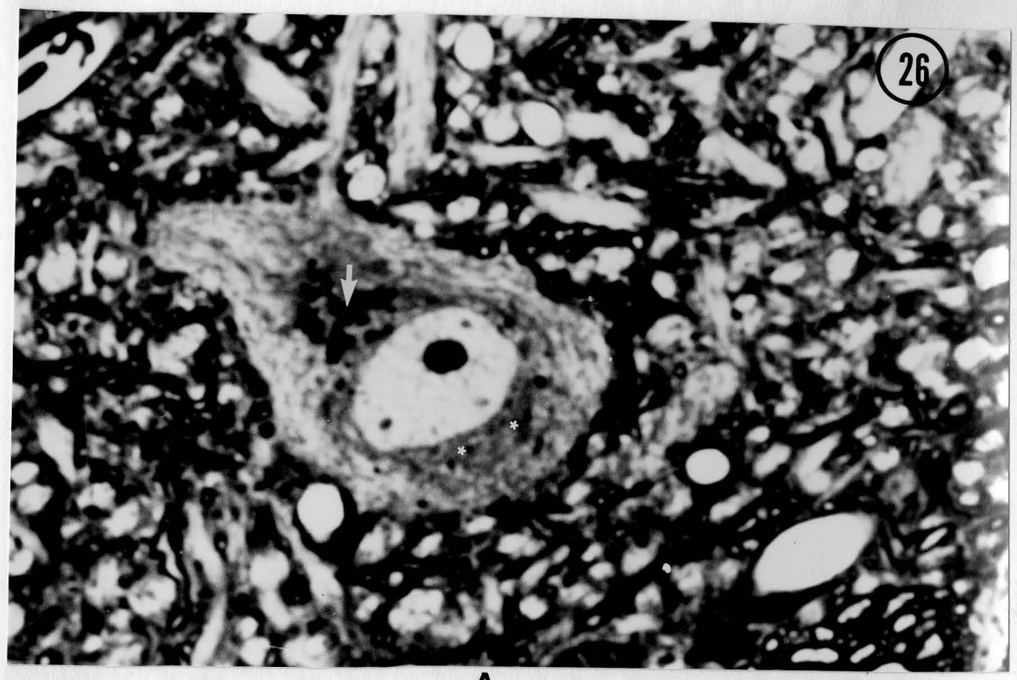


FIGURE 26

Comparison of normal and experimental facial motor neurons 5 days after axotomy in the adult hamster. Arrors indicate lipofuscin. Plastic section. Toluidine blue. (1440X)

A. Normal neuron. * = clumped Nissl substance.

B. Experimental neuron. Note the paler cytoplasm, the scattered lipofuscin, and eccentric nucleus.



A

B

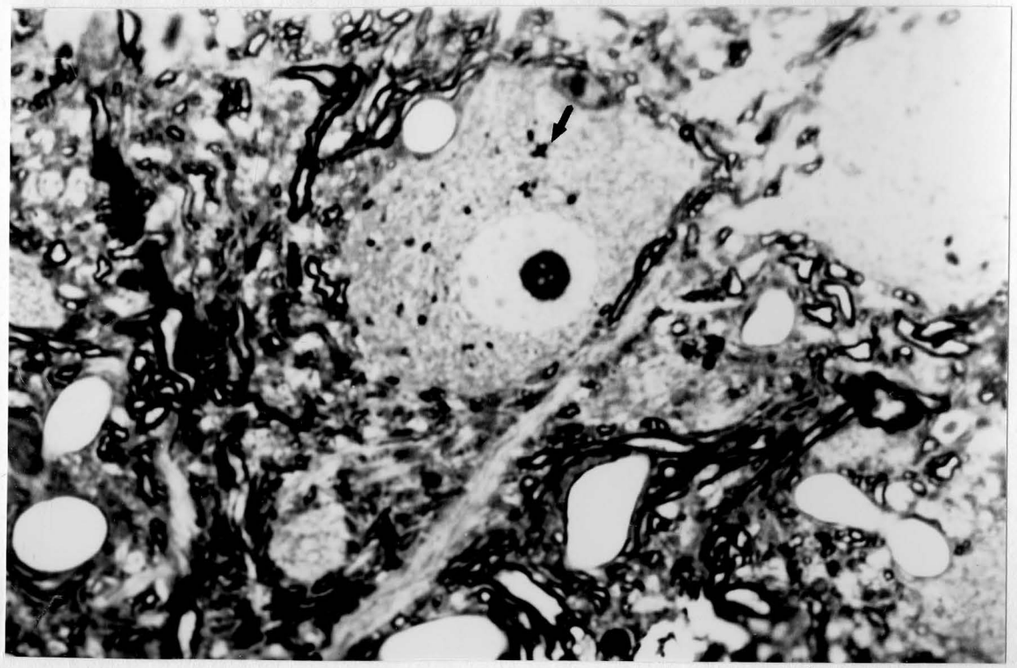


FIGURE 27

Representative cell types found in the normal and experimental facial motor nucleus of the adult hamster. Note that some oligodendrocytes lie near the neurons in a satellite position. Plastic section. Toluidine blue. (1440X)

A. Experimental side. 5 days after axotomy in the adult.

B. Normal side. MP = group of myelinated processes.

C. Normal side.

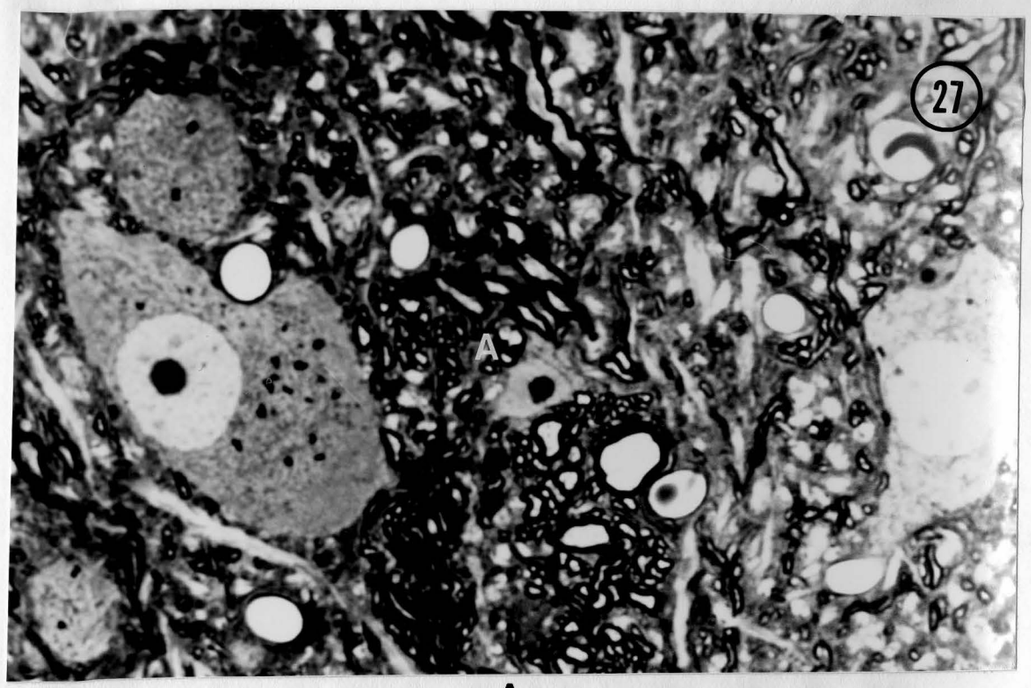
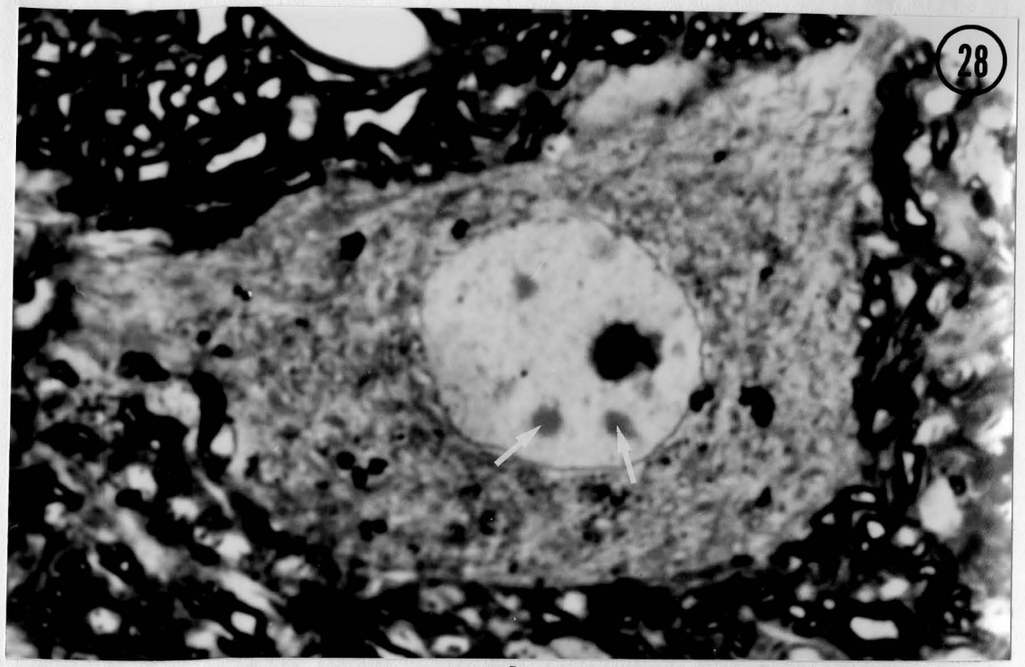


FIGURE 28

Detail of nuclear features typical of both normal and experimental facial motor neurons. Adult. Plastic section. Toluidine blue. (2800X)

A. Arrows indicate 'pink spots'.

B. Arrows indicate nuclear invaginations. Note oligodendrocytes.



A

B

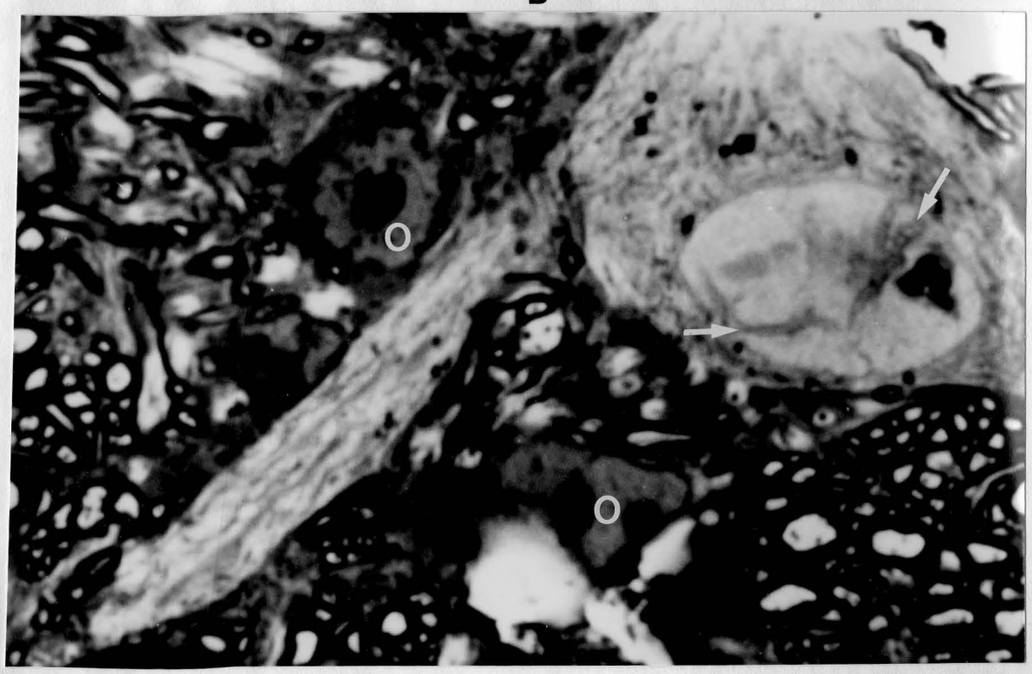


FIGURE 29

Electron micrograph of facial motor neuron from normal adult hamster. Bar = 1 micron. (5900X)

Arrow = Nucleolus

L = Lipofuscin

G = Golgi

RER = Rough endoplasmic reticulum

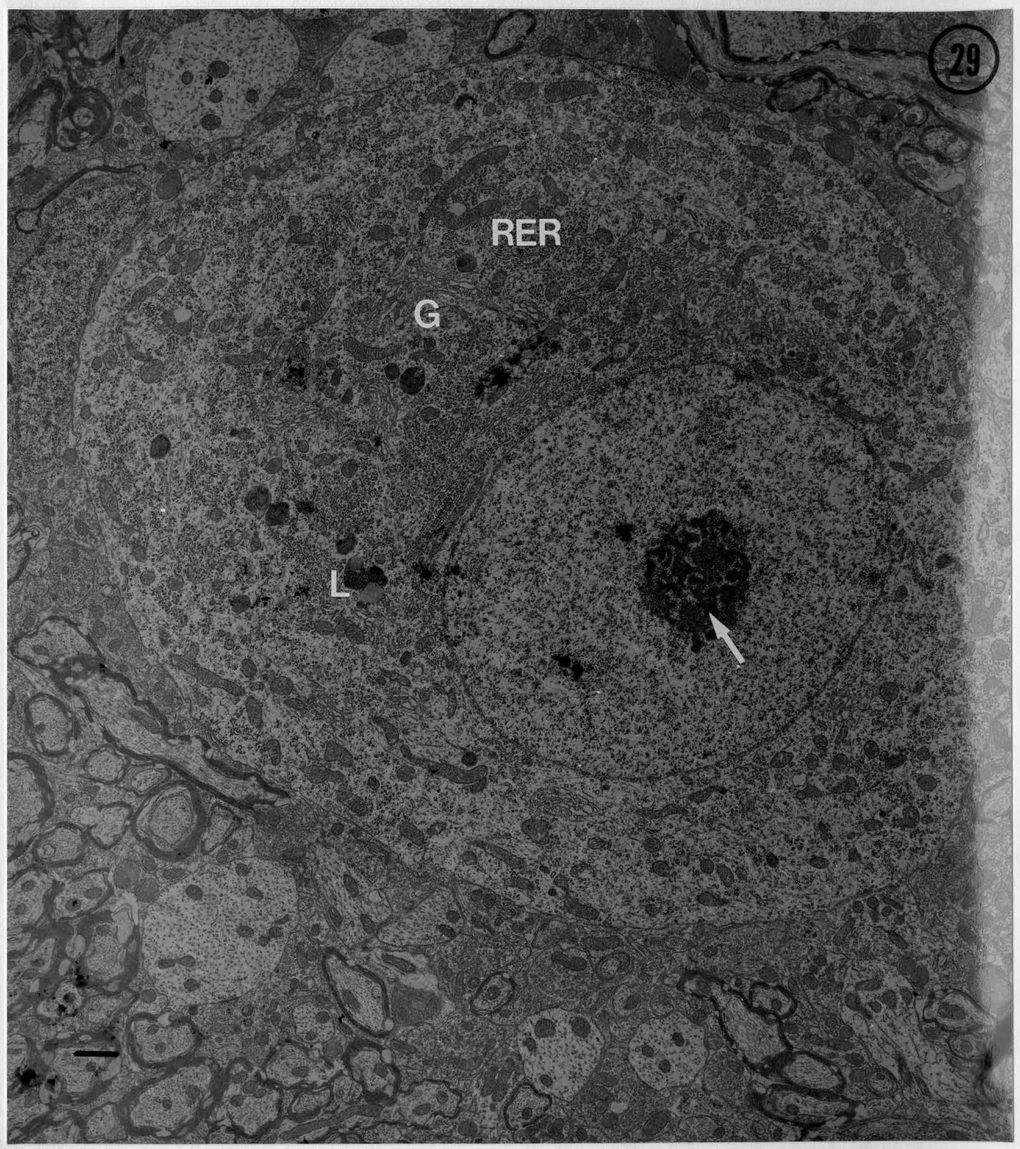


FIGURE 30

Electron micrograph of adult facial motor neuron in contact with an oligodendrocyte. Adult. Bar = 1 micron. (15,500X)

Ns = Neuronal soma

O = Oligodendrocyte nucleus

T = Microtubules in oligodendrocyte cytoplasm

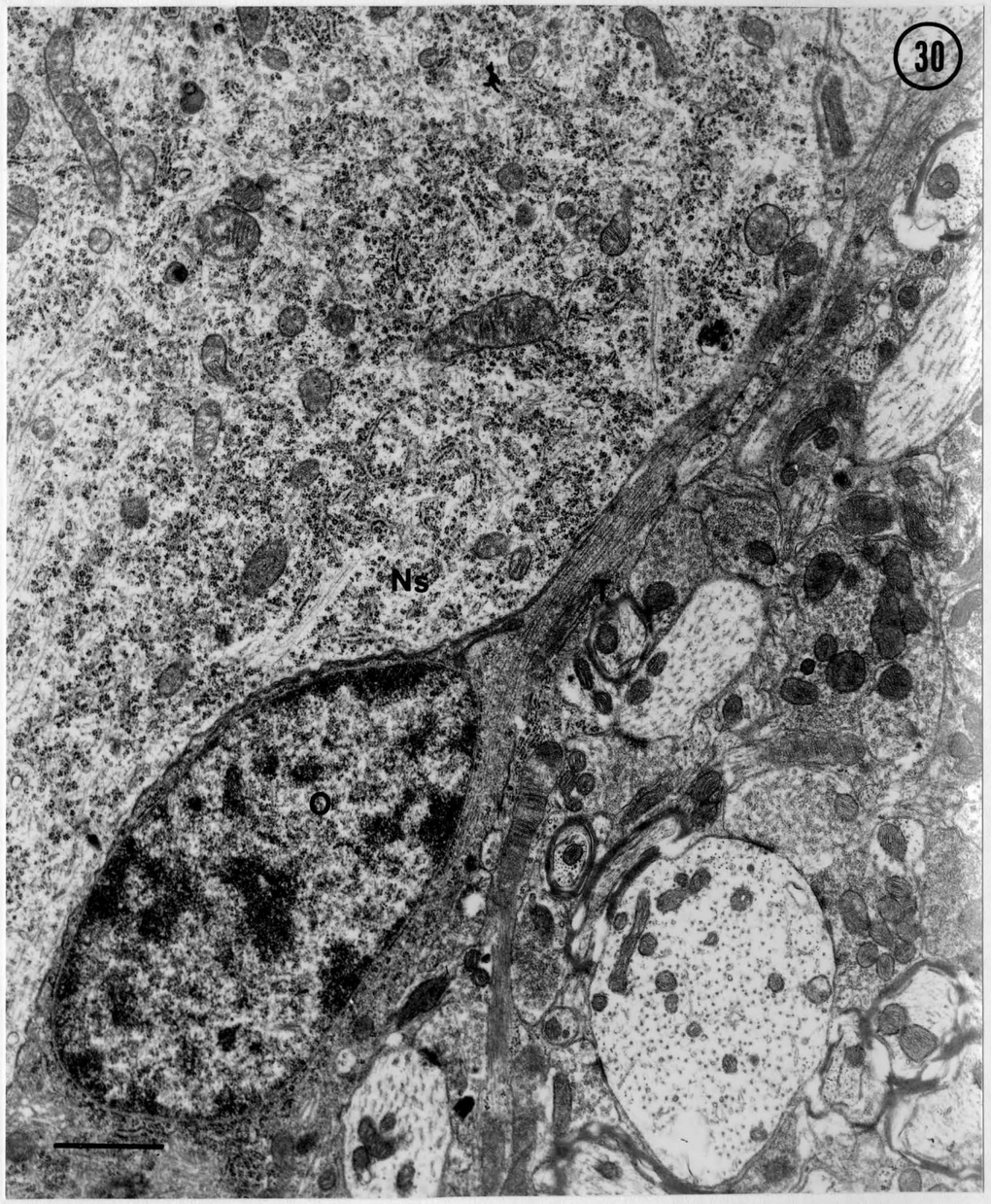


FIGURE 31

Electron micrograph of two oligodendrocytes in apposition to a neuronal soma (Ns). Adult. Bar = 1 micron. (12,700X)

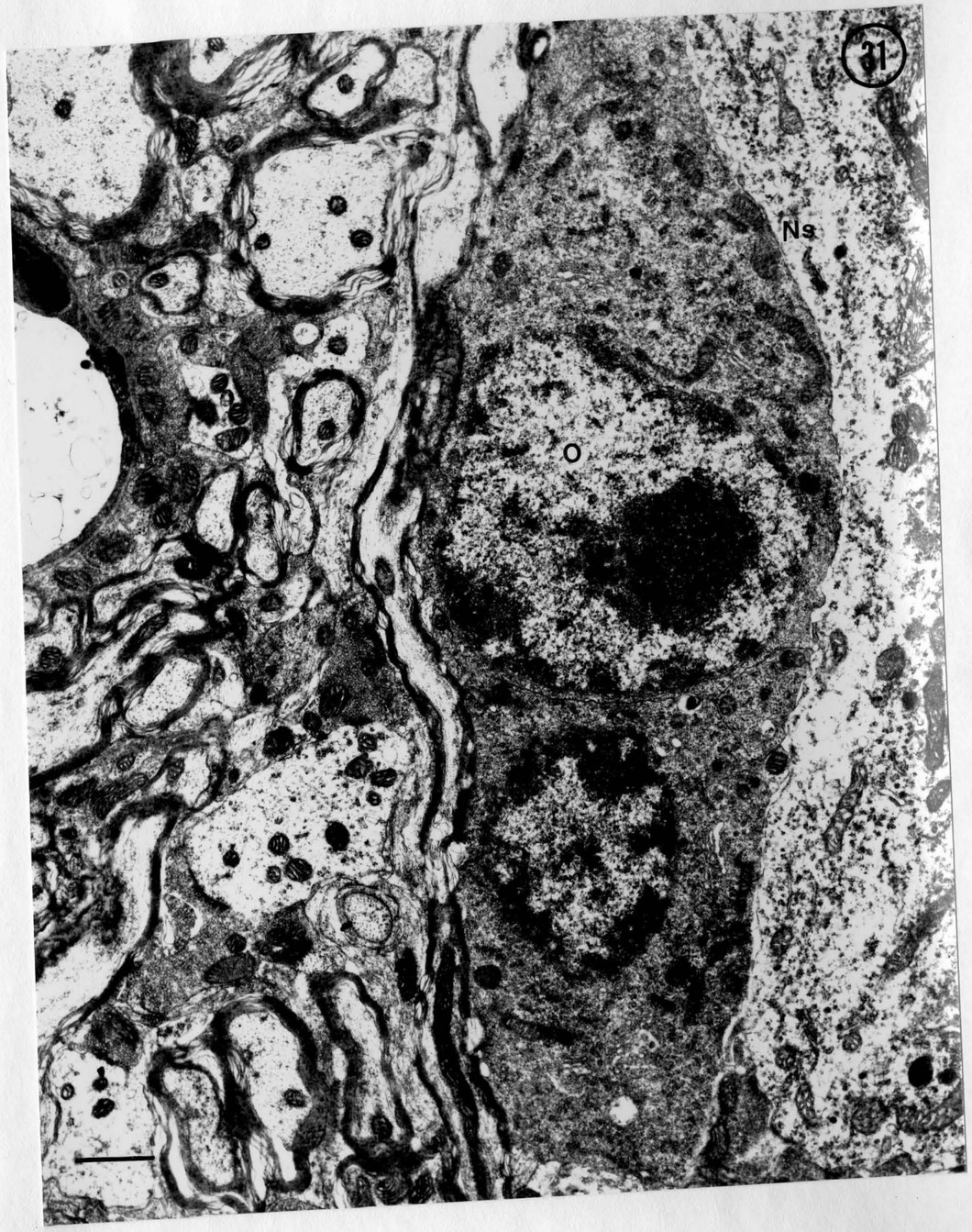


FIGURE 32

Electron micrograph of two oligodendrocytes in the facial motor nucleus of the adult hamster. Note their typical chromatin pattern. These cells are classified as 'light' type. (Compare with Fig. 33.) Bar = 1 micron. (11,300X)

32

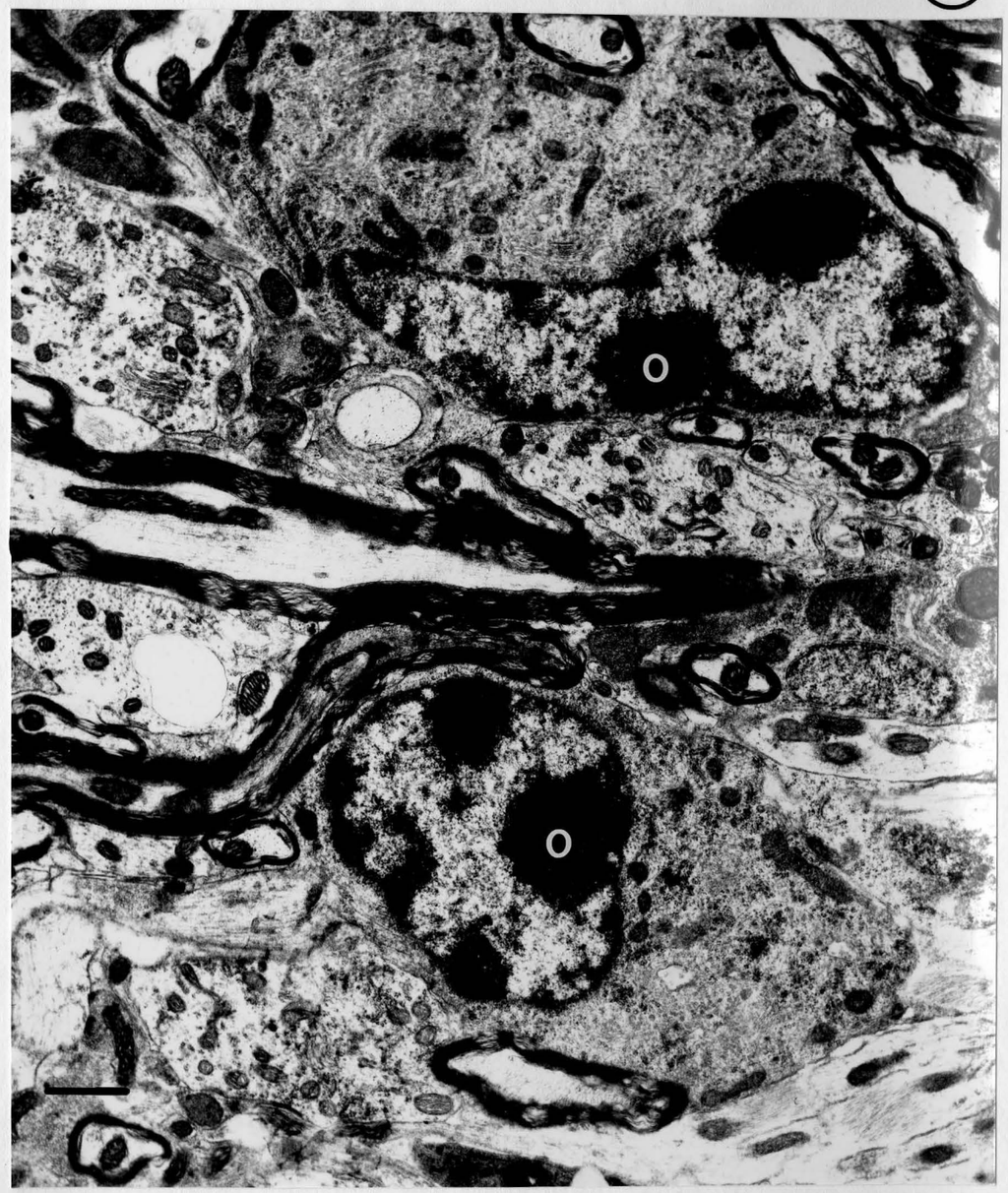


FIGURE 33

Electron micrograph of a dark oligodendrocyte in the facial motor nucleus of the adult hamster. (Compare with Fig. 32.) Arrows = RER. Bar = 1 micron. (12,700X)

33

FIGURE 34

Electron micrograph of astrocyte in the facial motor nucleus of the adult hamster.

A. Astrocyte showing nucleus with typical single chromatin mass. Bar = 1 micron. (11,300X)

B. Detail of cytoplasmic filaments (arrow). Bar = .25 micron. (79,800X)
Note many large, myelinated processes (MP) in the field.

A

B

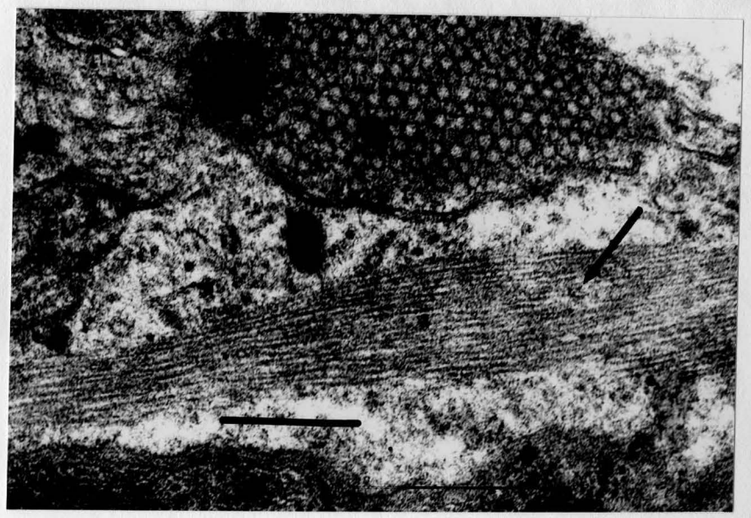


FIGURE 35

Electron micrograph of 2 astrocytes in the facial motor nucleus of the adult hamster. Arrows indicate cytoplasmic filaments. Bar = 1 micron. (12,500X)

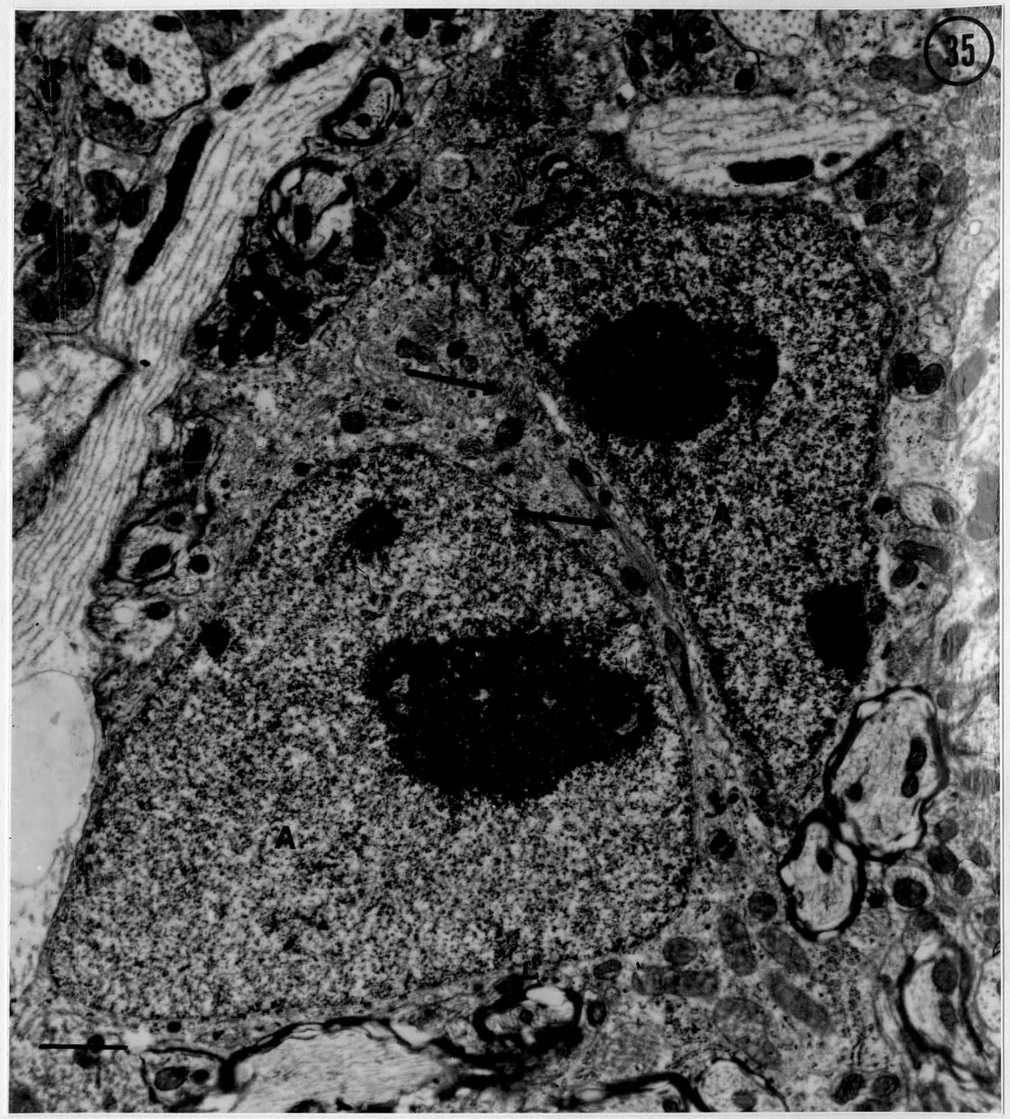


FIGURE 36

Electron micrograph of facial motor neuron 4 days after axotomy in the adult hamster. Note the scattered ribosomes and lack of stacked RER throughout the cytoplasm. Nc = Nucleus. Bar = 1 micron. (5400X)

36

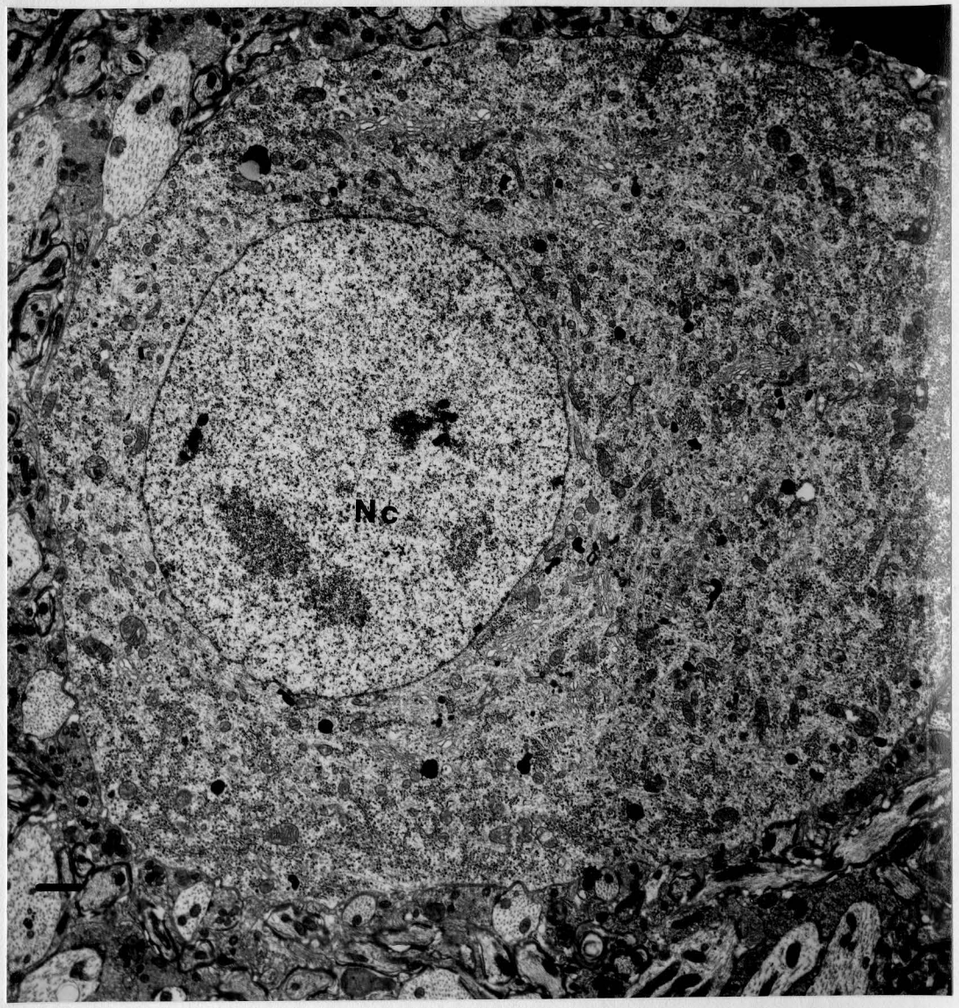


FIGURE 37

Electron micrograph of facial motor neuron 100 days after axotomy in the adult hamster. Note the return of stacked RER (arrows). (Compare with Figs. 29 and 36). Nc = Nucleus. Bar = 1 micron. (6300X)

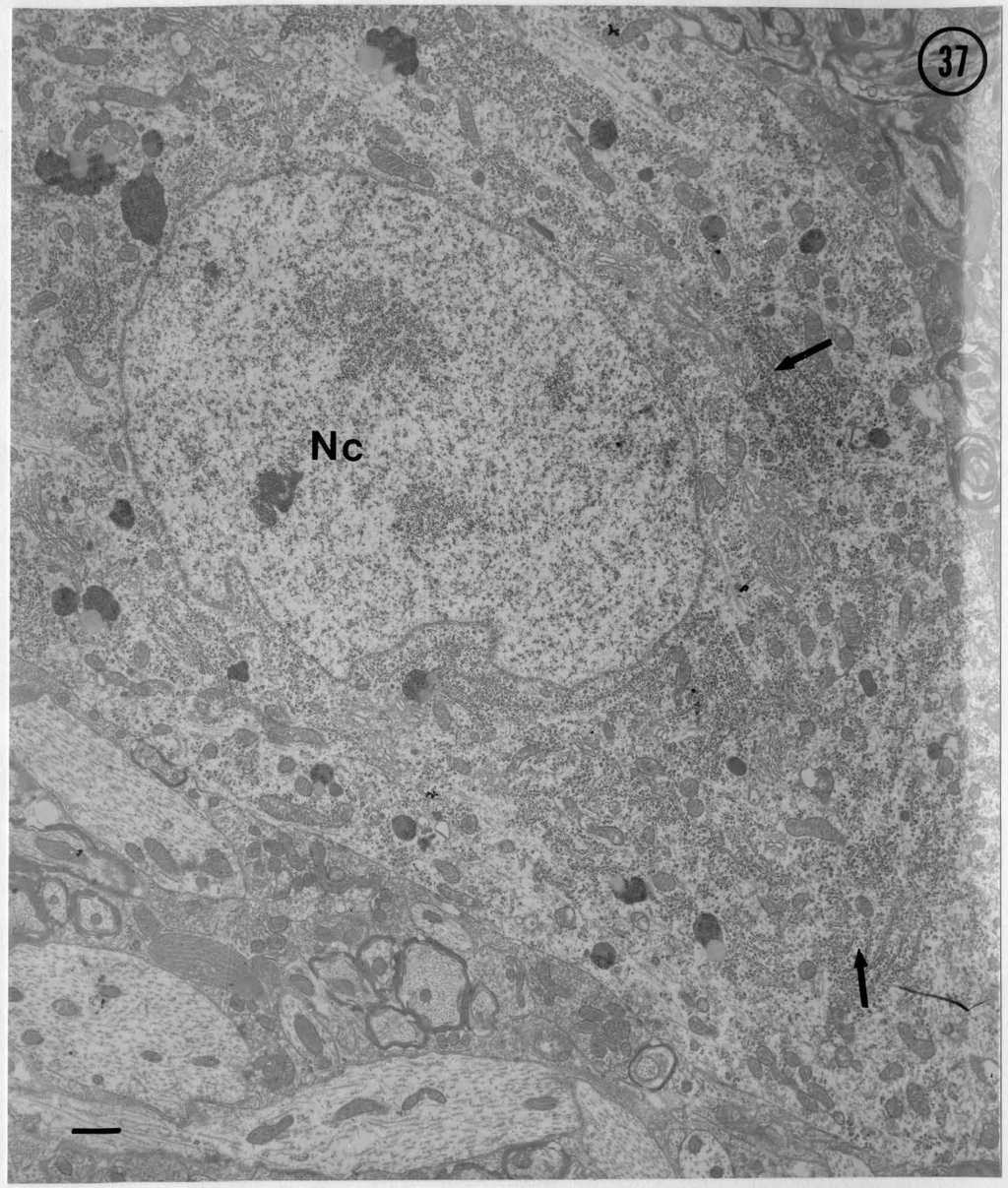
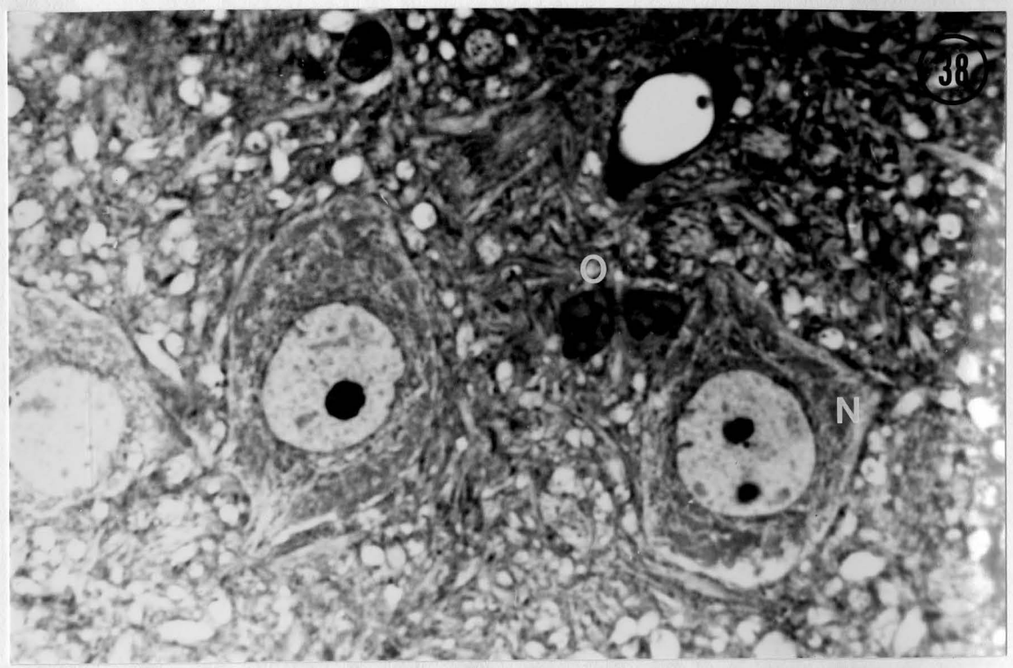


FIGURE 38

Two views (A and B) of representative cell types found within the facial motor nucleus of the 4 day old normal hamster brain. Note the lack of myelinated processes. (Compare with adult, Fig. 27.) Plastic section. Toluidine blue. (1440X)



A

B

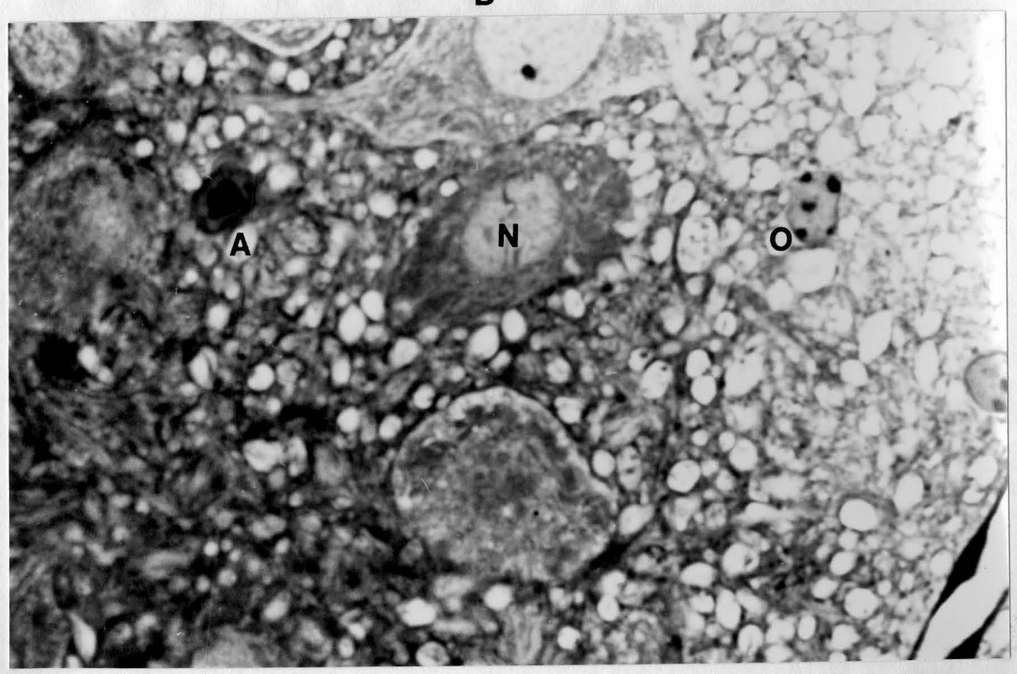


FIGURE 39

Electron micrograph of a facial motor neuron of the 4 day old normal hamster brain. Arrows indicate stacked RER. Nc = Nucleus. Bar = 1 micron. (7000X)

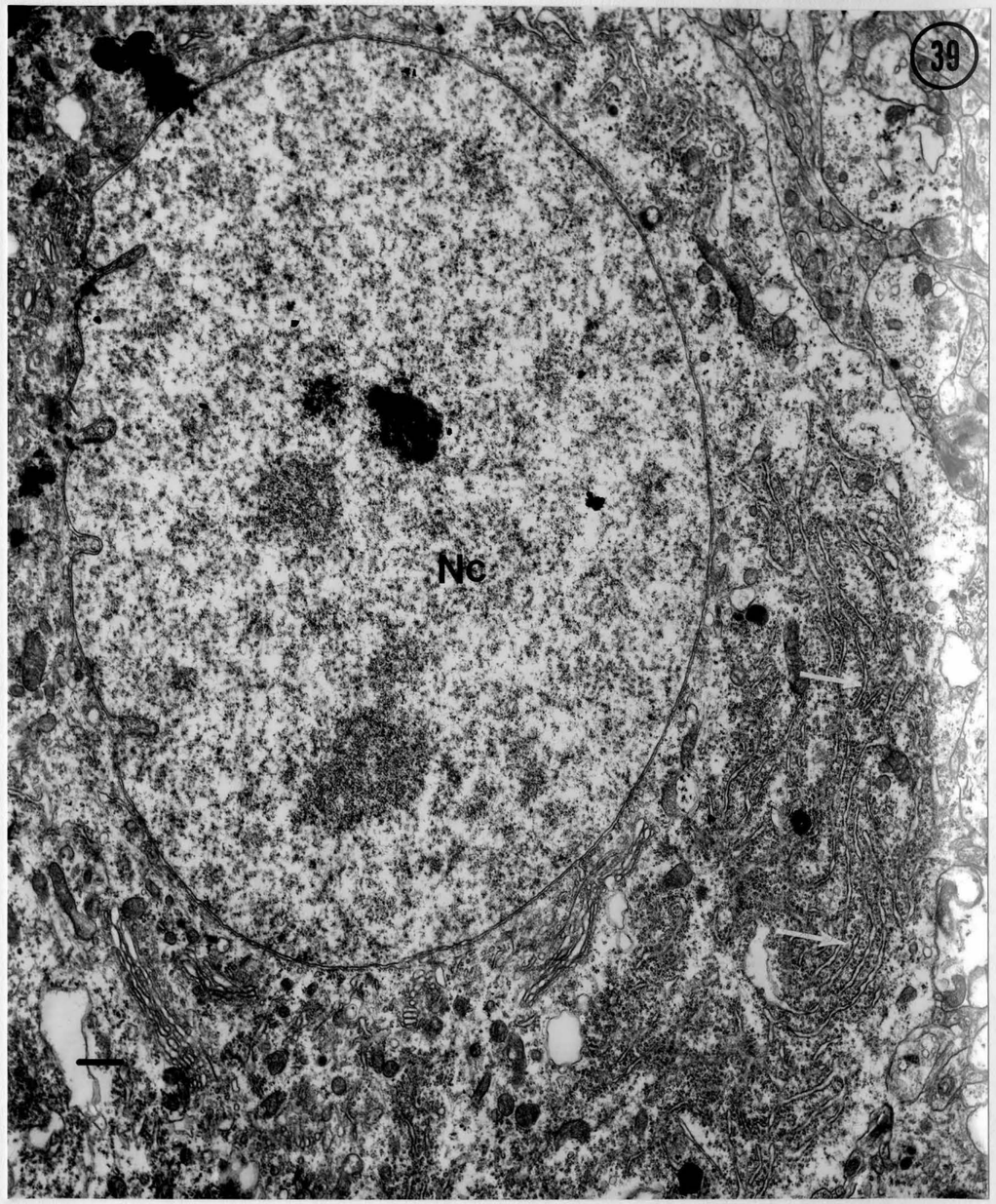


FIGURE 40

Electron micrograph of a facial motor neuron of the 4 day old hamster brain. Nc = Nucleus. * = Nuclear invaginations. Bar = 1 micron. (14,100X)

FIGURE 41

Electron micrograph of a facial motor neuron of the 4 day old hamster brain. Nc = Nucleus. Arrow = Stacked RER. * = Very large nuclear invagination. Bar = 1 micron. (14,100X)

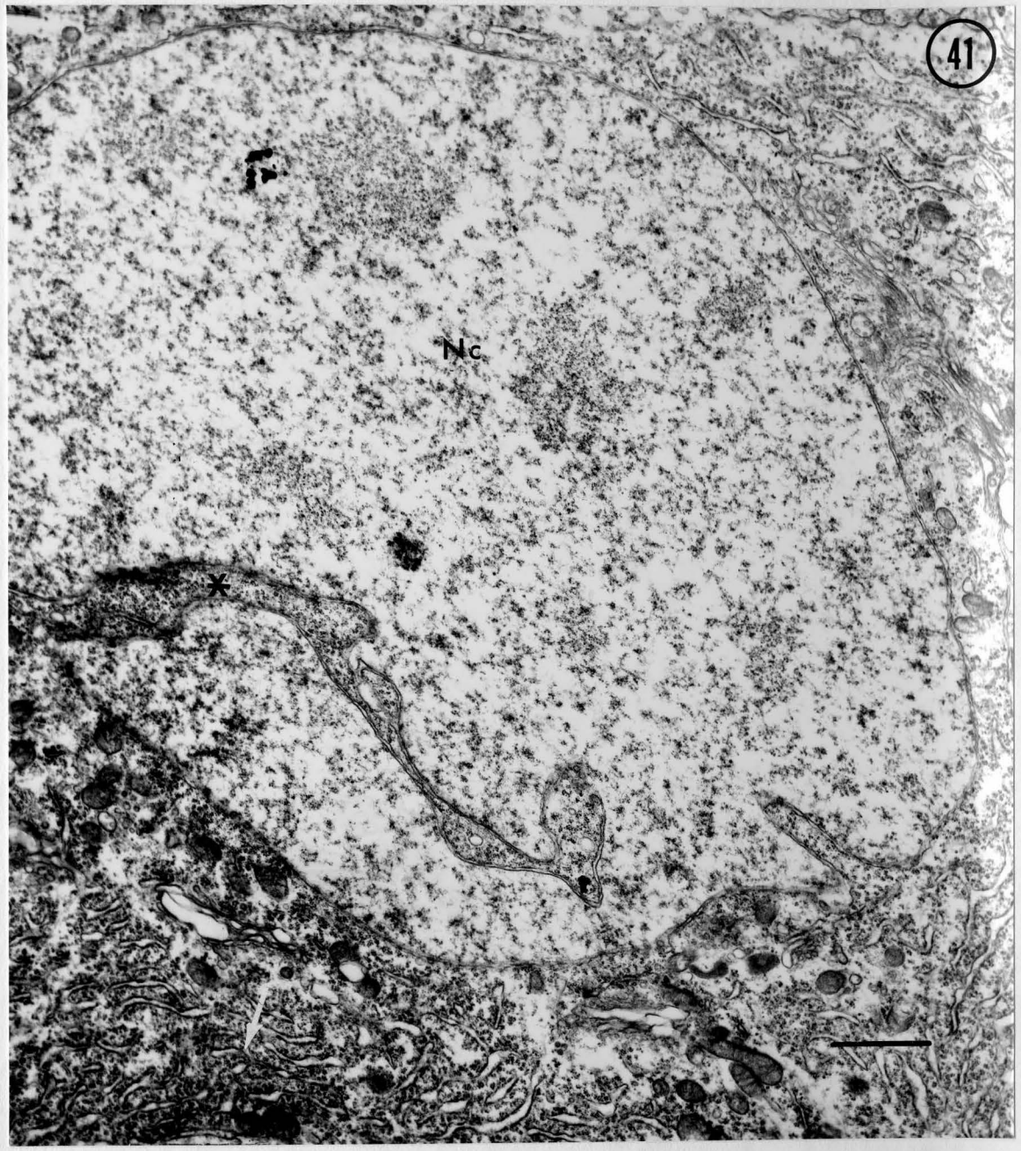


FIGURE 42

Electron micrograph of glial cells in the facial motor nucleus of the 4 day old normal hamster. Note typical chromatin patterns. Bar = 1 micron.

A. Oligodendrocyte near a neuron. Ns = Neuronal soma. Note relative lack of cytoplasmic organelles in glial cytoplasm (compare with adult, Figs. 30-33). (10,200X)

B. Astrocyte. Note group of very small, unmyelinated processes (P) and compare with myelinated processes of the adult (Figs. 33 and 34). (12,500X)

A

B

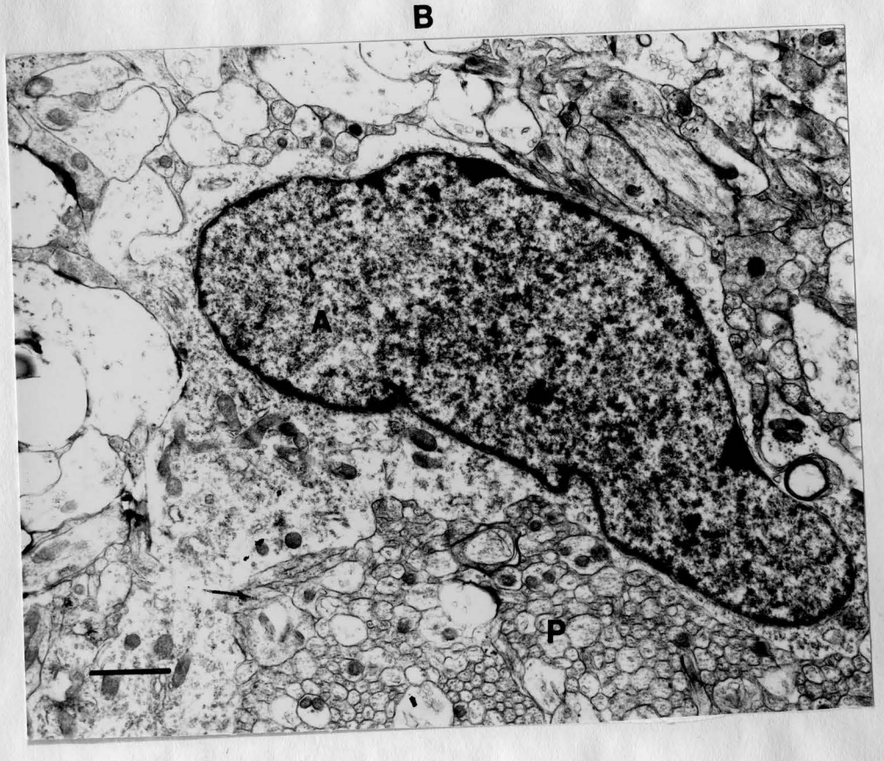


FIGURE 43

Electron micrograph of glial cells in the facial motor nucleus of the 4 day old normal hamster. Note the typical chromatin patterns of the cell nuclei, as well as the 'blunted' extension of astrocytic cytoplasm (As). Bar = 1 micron. (6900X)

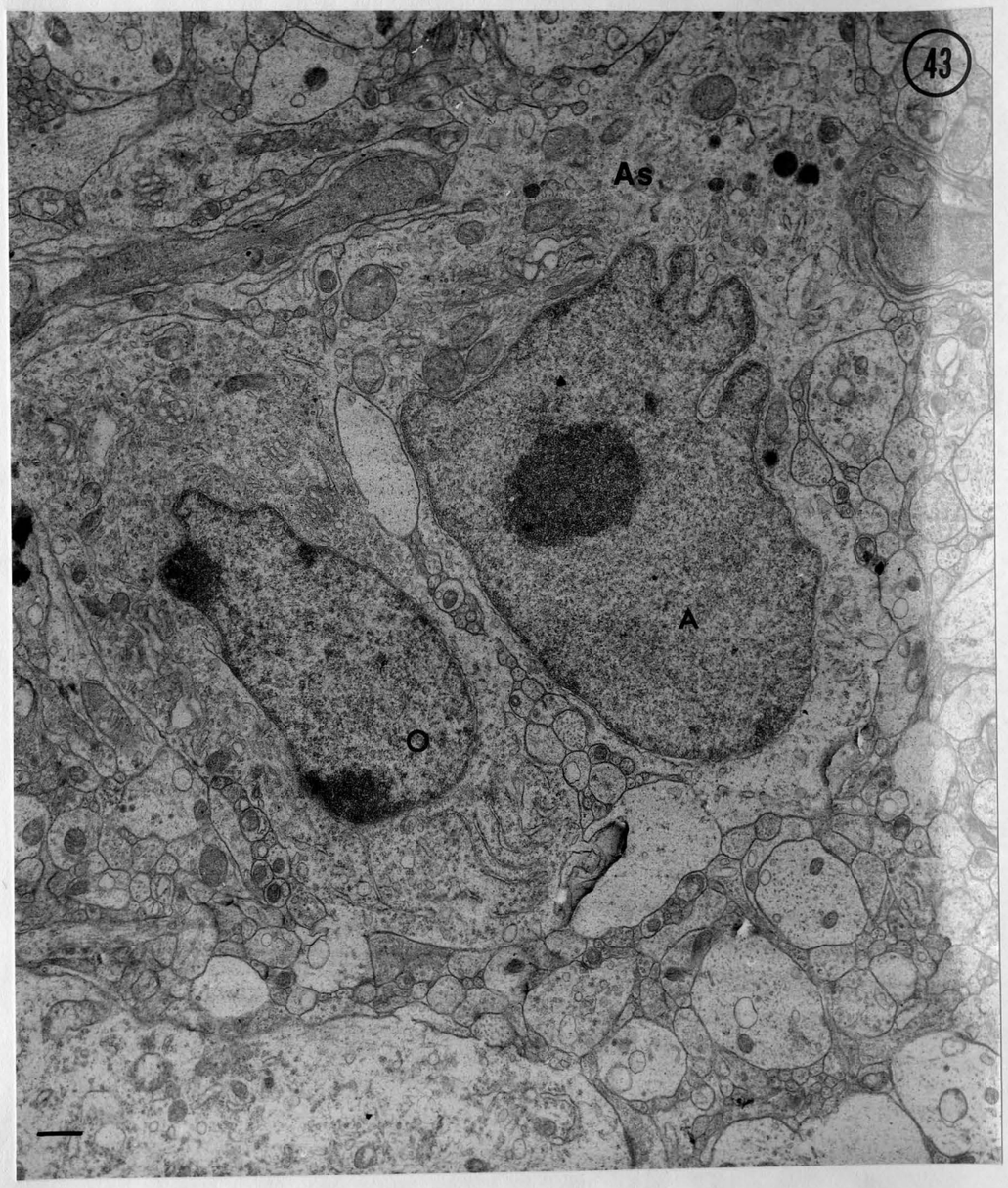


FIGURE 44

Representative cell types found 3 days after axotomy at 1 day of age. Note appearance of dark staining debris (arrow). * = Dying neuron. Plastic section. Toluidine blue. (1440X)

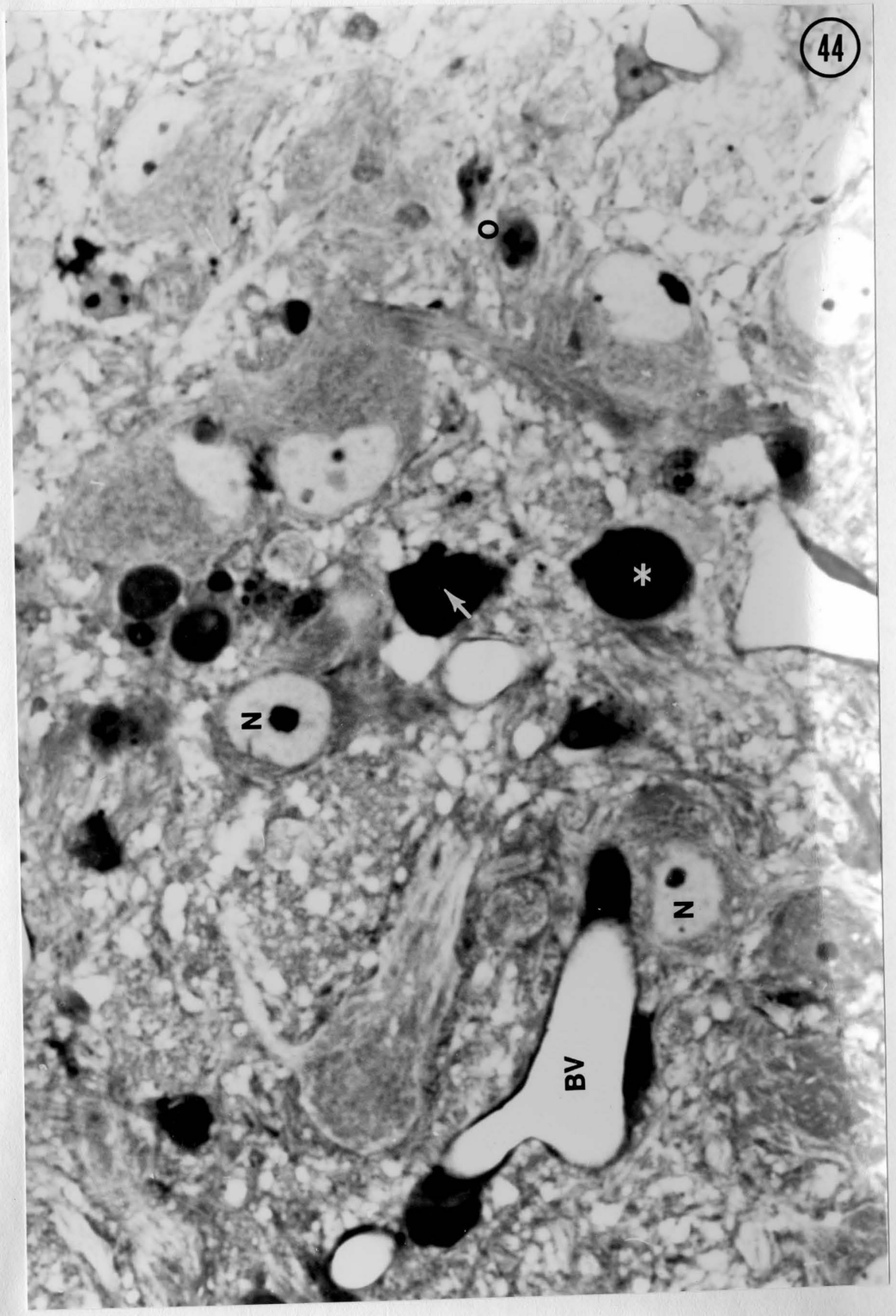


FIGURE 45

Representative cell types found 3 days after axotomy at 1 day of age. Note appearance of dark staining debris (arrow). Plastic section. Toluidine blue. (1440X)

FIGURE 46

Electron micrograph of a facial motor neuron 3 days after axotomy at 1 day of age. Note extreme dilution of cytoplasm. Arrows indicate pinching off of processes. Bar = 1 micron. (15,700X)

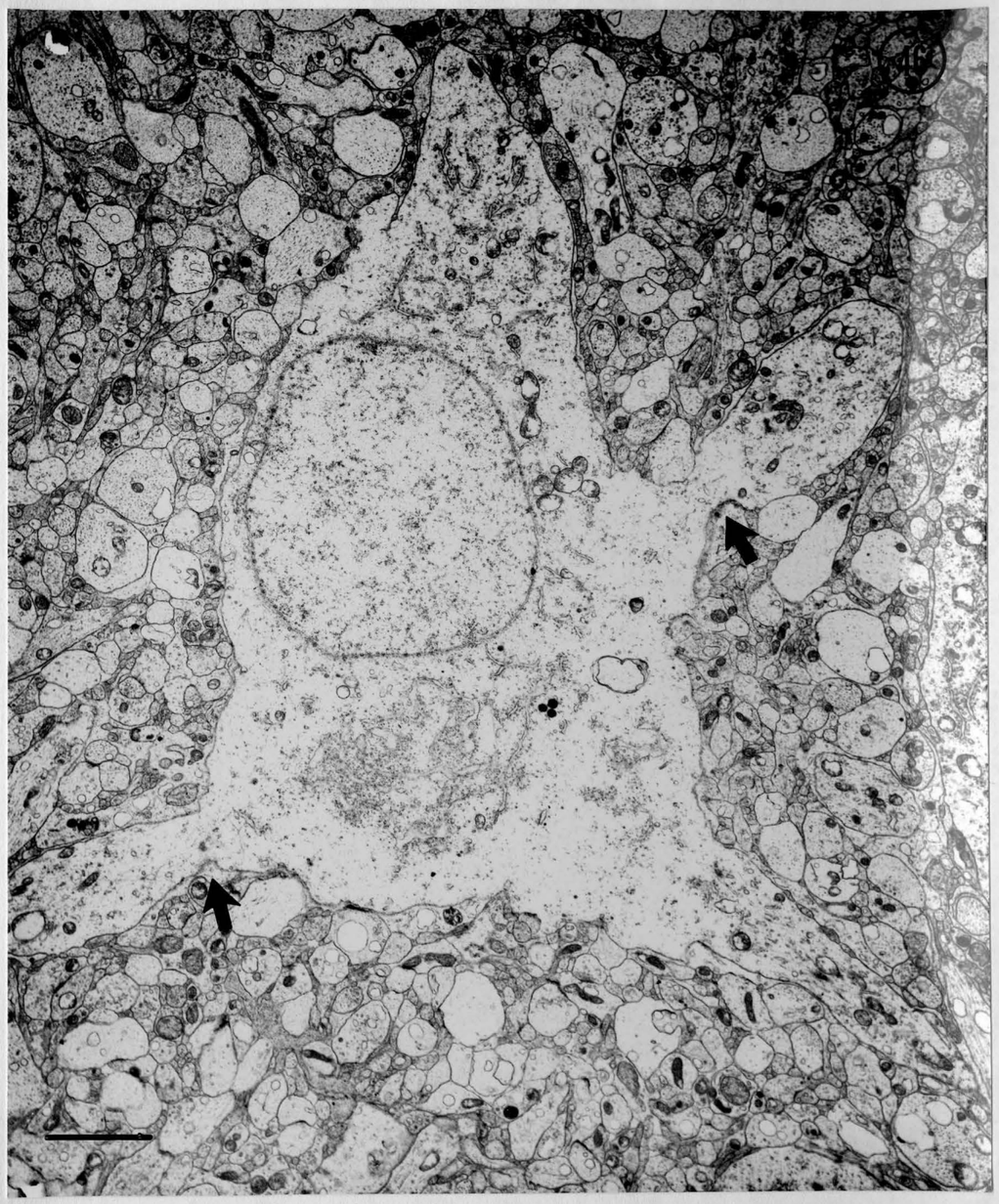


FIGURE 47

Electron micrograph of a facial motor neuron 3 days after axotomy at 1 day of age. Note presence of RER in the cytoplasm; also, autophagic vacuoles and dense bodies (arrows). Bar = 1 micron. (6300X)

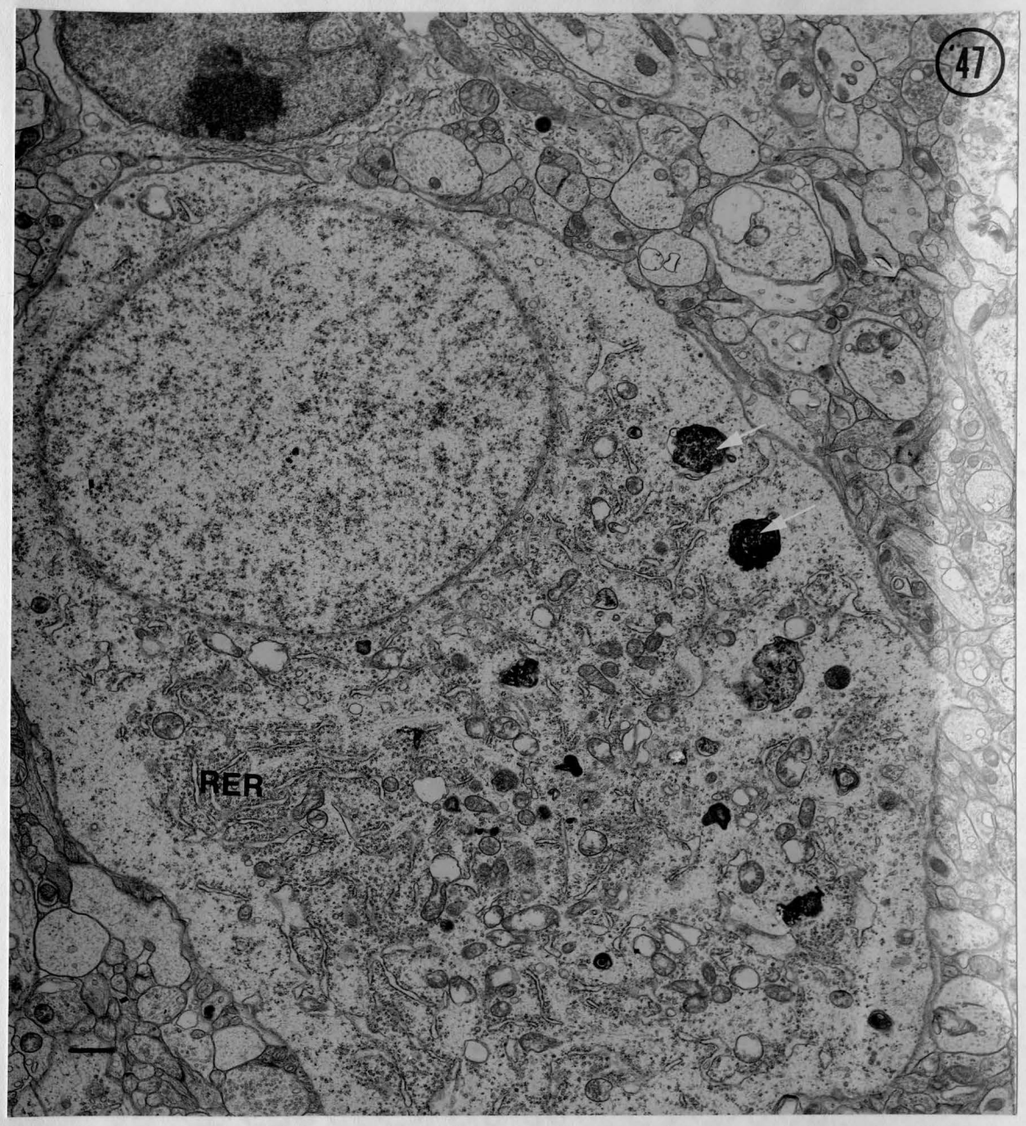


FIGURE 48

Electron micrograph of oligodendrocytes near some neuronal debris (arrows) in the facial motor nucleus 3 days after axotomy at 1 day of age. Bar = 1 micron. (17,300X)

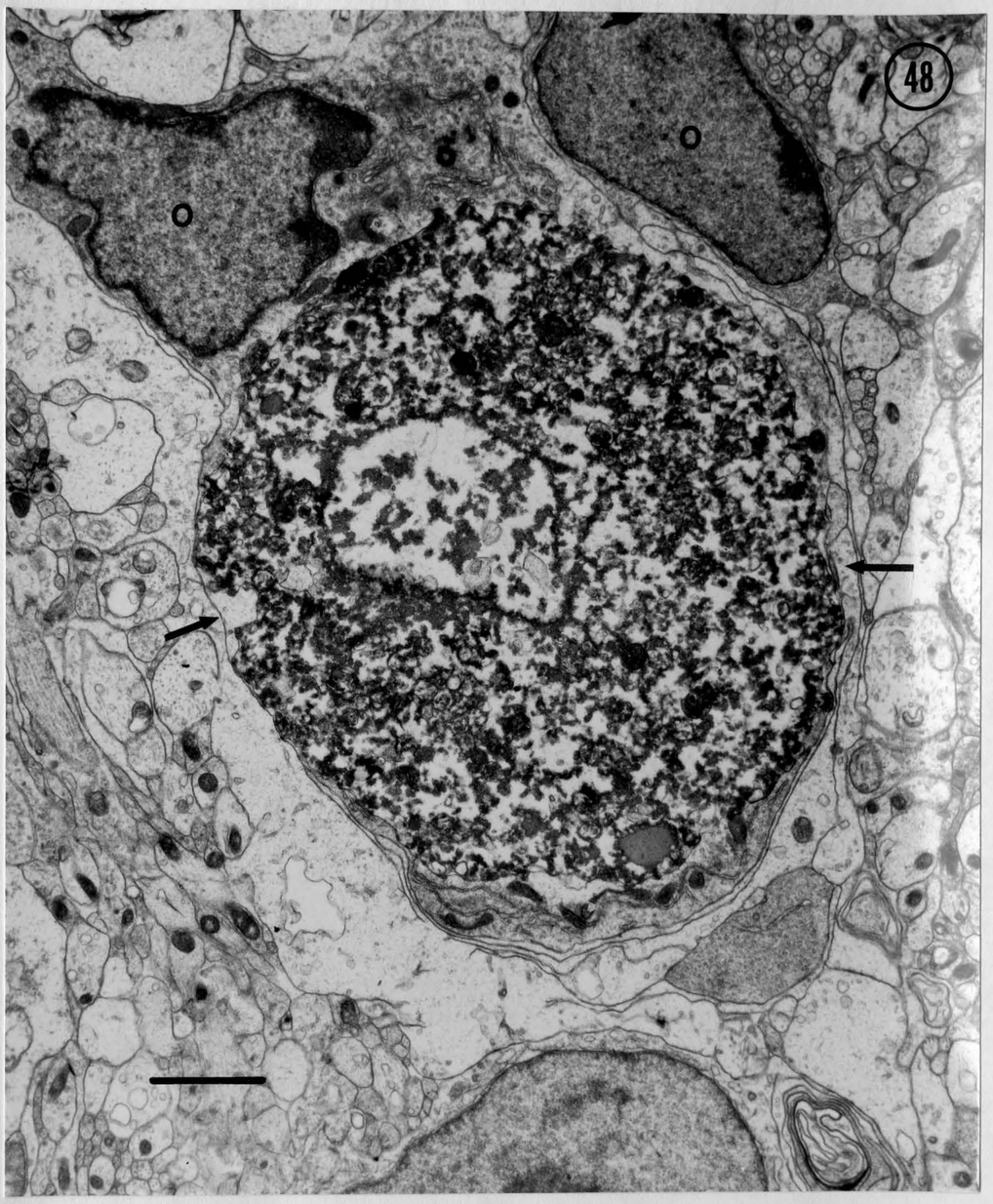


FIGURE 49

Electron micrograph of oligodendrocyte near a facial motor neuron 3 days after axotomy at 1 day of age. Ns = Neuronal soma. Bar = 1 micron. (7000X)

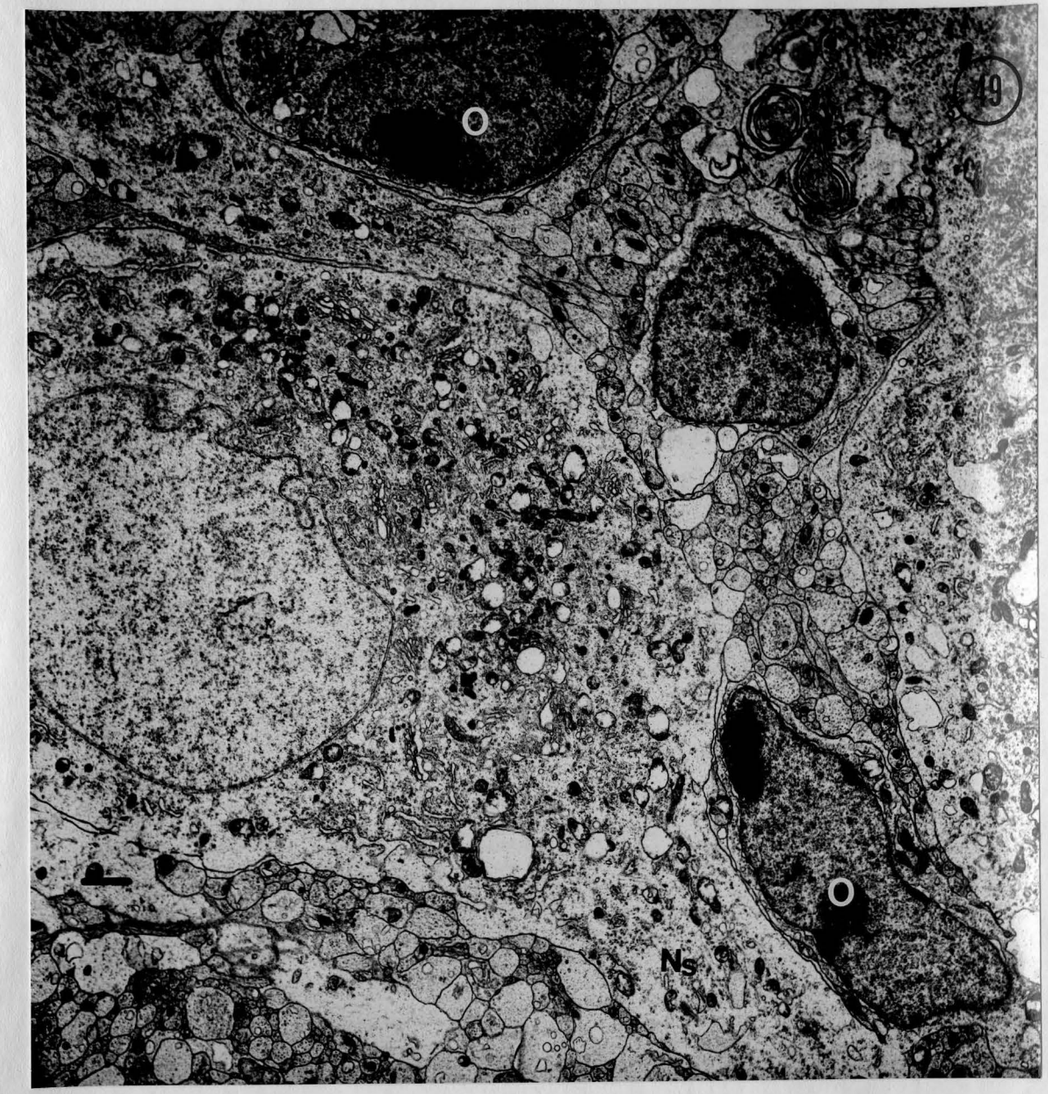
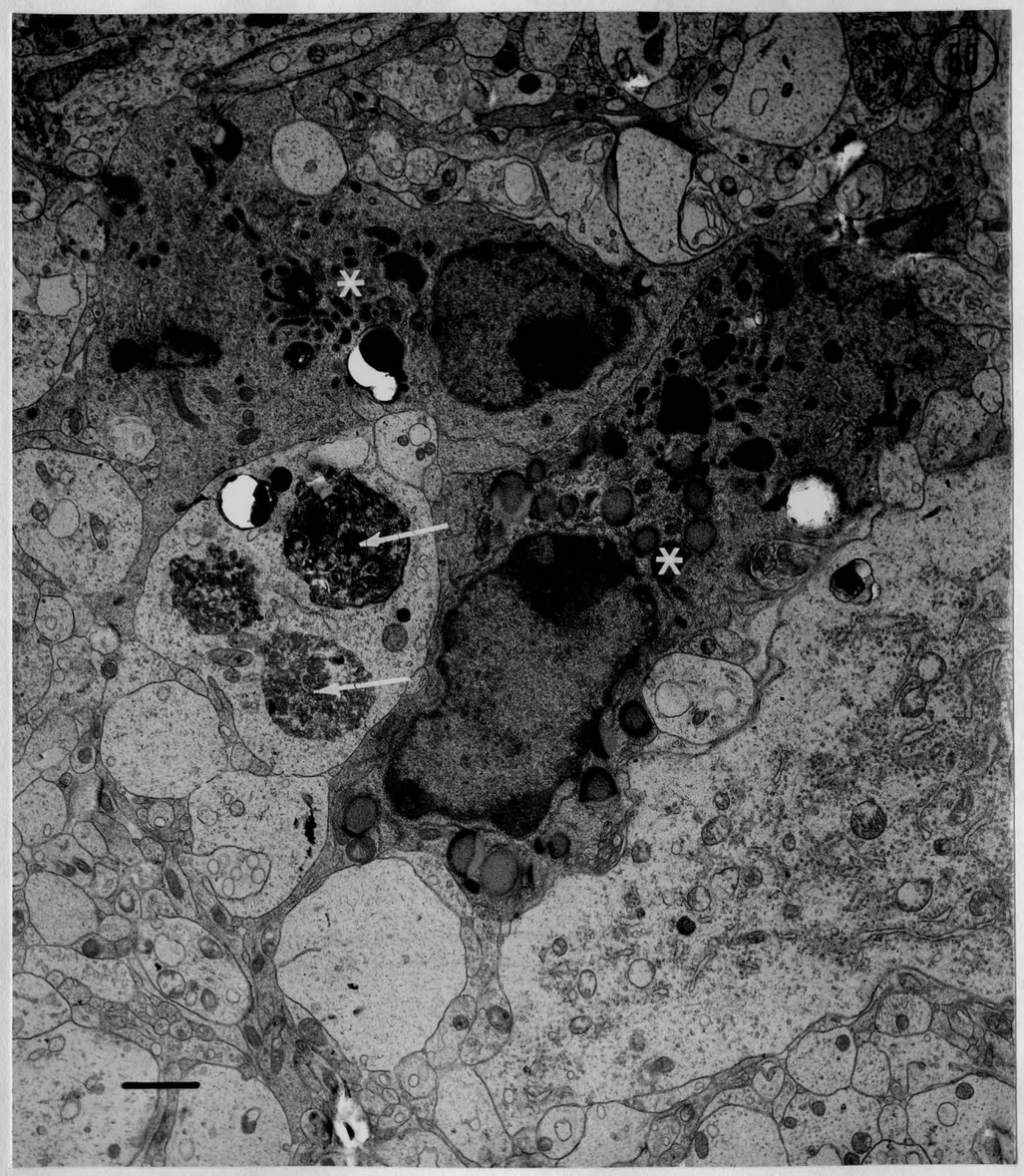


FIGURE 50

Electron micrograph of reactive cells near neuronal debris in the facial motor nucleus 3 days after axotomy at 1 day of age. Note the similarity of their morphology with oligodendrocytes and microglial cells. Bar = 1 micron. Arrows = dense bodies; * = debris. (11,300X)



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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and the dissertation is now given final approval by the committee with reference to content and form.

The dissertation is therefore, accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 15, 1978
Date

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Director's Signature